AMENITIES AND DEVELOPMENT IN BRITAIN

ARTIFICIALLY preserved natural beauty, R. G. Collingwood once pointed out, is a contradiction in terms; but even the most casual reading of the annual reports of the National Parks Commission, the Nature Conservancy or the National Trust shows how positive is the conception of preservation and conservation which those bodies seek to promote in Britain. Ten years of work by the Nature Conservancy, for example, has demonstrated how vital a part conservation has to play in Britain's economy; and the work of the National Parks Commission equally attests the soundness of the original conception of a national park. From the start, the National Parks Commission has held firm the concept expressed by the late John Dower in his report in 1945, that the fundamental decision made where a given area is established as a national park is that within that area two dominant purposes, out of the numerous possible ways in which the land might be used and developed, shall thereafter stand supreme; all other uses, though by no means excluded, being so limited and controlled as to harmonize with the two dominants. These two purposes are that the characteristic beauty of the landscape shall be preserved and that the visiting public shall have ample access and facilities within it for open-air recreation and enjoyment of its beauty.

In its first report, the National Parks Commission made it plain that these concepts governed its attitude to development within national parks, although it recognized equally that the nation's economy and safety might not be able to afford the entire exclusion from the parks of such activities as quarrying, forestry, water catchment, electricity schemes or defence measures. Similar considerations apply no less to the Nature reserves, and here the work of the Nature Conservancy has increasingly shown the value of conservation to the nation's economy, so that even from that point of view conservation in such areas is the over-riding interest. Nevertheless, despite the utmost endeavours of the Commission and the Conservancy to meet demonstrated needs of development, it is impossible to cite a single instance during the past ten years when within the national parks or Nature reserves the preservation of natural beauty or the conservation of flora or fauna has been recognized, in the face of other demands, as the supreme national interest. The Government's decision has invariably been that amenity or conservation can be sacrificed, and commonly in circumstances which justify some scepticism as to whether the national, rather than a sectional, interest is being allowed to prevail.

It is against this background, and particularly after such a decision as that taken regarding Dungeness last year, that the latest decision to establish a ballistic missile early warning station at Fylingdales Moor in the North Yorkshire Moors National Park arouses such profound misgivings. The decision was announced in the House of Commons on February 17 by the Secretary of State for Air, Mr. G. Ward, who said that the station was the outcome of agreement with the Government of the United States of America and in support of the North Atlantic Treaty. The station, which would be on Government-owned land, would be commanded and operated by the Royal Air Force, and the information it obtained would be available simultaneously to operation centres in the United Kingdom and the United States. The capital cost to Great Britain, which would provide the land, erect the buildings, provide the communications required to link the station with the British authorities, and maintain the equipment, was expected to be about £8 million, and to the United States, which would provide and install the radar equipment and pay for the communications required to link the station to the rest of the system, about £35 million. After the first five years, the responsibility for the costs of maintenance would be reviewed. In reply to questions, Mr. Ward said that the uniformed man-power would amount to about 50 officers and men; about 2,000 persons would be employed on building, and eventually employment would be available for 200–300 people.

Mr. Ward added that the Government greatly regrets that the station has to be in part of a national park, but the topographical, geographical and operational criteria governing the choice of site were stringent, and the Government is satisfied that there is no other suitable site in the whole country. The Fylingdales site comprises about four square miles of land, owned mainly by the War Office and partly by the Ministry of Agriculture on behalf of the Forestry Commission, and it is within a larger area which has been used by the War Office for military training. Mr. Ward also said that the Air Ministry will discuss with the local authorities, and with the National Parks Commission and other amenity bodies, the detailed siting arrangements and measures to be taken to minimize the effect on the landscape. Challenged by Mr. de Frietas that once again a national park has to provide the only possible site in the whole of Britain, Mr. Ward repeated that the last thing he wished to do was to put the station in a national park, but no suitable alternative site in Britain could be found.

A parallel statement was made in the House of Lords on the same day by the First Lord of the Admiralty, Lord Carrington, who indicated that it is intended that about 500 people will operate the station. In reply to Lord Alexander of Hillsborough, who had challenged the choice of a site in a national park, Lord Carrington, while sympathizing with what Lord Alexander had said, claimed that the Ministry of Housing and Local Government is convinced that this is the only possible site to use. The station must be in a very thinly populated area, and any other site

would have been open to even greater objections than this one is. Some of the requirements to be met had, of course, to be kept secret.

Although in opening a debate on electrical development and landscape beauty in the House of Lords on February 25, Lord Chorley made passing reference to the proposed radar station at Fylingdales, the project was not otherwise considered in that debate; although apart from two special considerations, namely the magnitude of the scientific effort involved in this investment of more than £40 million and the possible repercussions of this installation on bird life, the project is most appropriately considered in the context of development in general. Both the recent annual report of the National Parks Commission and that of the Nature Conservancy recognized that development is the major threat to their work, and while the Commission can point to numerous instances where its advice has led to less damage to amenity, the assurance which the report quotes from the Minister of Housing and Local Government's speech at Bakewell on May 29 would be more convincing if it were possible to point to a single major instance when the decision had been in favour of amenity and against a State department.

The question of the most appropriate use of land is fast becoming a critical problem in Great Britain. It is accentuated by every fresh proposal for motorways and highway improvement, for re-housing and satellite towns, for hydro-electric schemes and water undertakings, and nuclear power stations; and frequently the secondary effects are as considerable as the direct. It has even been suggested that Britain needs a Ministry of Development and Conservation related to such departments as the Board of Trade, the Ministry of Agriculture, the Forestry Commission and the Ministry of Transport as is the Ministry of Defence to the three Service Ministries. The Council for the Preservation of Rural England was advised last October by Mr. J. R. James, deputy chief technical planning officer of the Ministry of Housing and Local Government, that the most effective way of preserving the most beautiful areas is to see that there is sufficient land set aside in the less-beautiful areas to meet all requirements which are likely to arise in the next twenty years.

Mr. James did not appear to be perturbed about the loss of some 30,000 acres of agricultural land every year to meet the needs of development; but he did suggest that we were not paying a high enough price for the preservation of amenity. He referred particularly to the voluntary acceptance of restrictions on individual freedoms; but that is no less true of the financial aspect, and the National Parks Commission believes with reason that the public would not be reluctant to spend 6*d*. a person a year for the preservation and enhancement of our national heritage in the country, in place of less than 1*d*. as during the past ten years. How that expenditure is to be provided is another matter, whether through an adequately financed National Parks Commission or by the electricity or other authorities which may disturb amenity; but at least it should be on the basis of common discussion and agreement leading to decisions where development should be encouraged and where it should be resisted or denied, and not piecemeal and *ex parte* judgments or decisions. As things are, it might well be thought that designation of an area as a national park, a Nature reserve or an area of outstanding natural beauty is almost certain to attract to that area some development proposal which will be decided against amenity or conservation.

It is interesting to note that at the dedication last May of the new buildings of the Rockefeller Institute, its president, Dr. D. W. Bronk, spoke of natural beauty and science, emphasizing the care which was being taken in the design of the new buildings in relation to the surrounding landscape, so as to provide a pleasant and beautiful environment, suitable for scholarly associations. In his address, Dr. Bronk quoted some remarks of G. M. Trevelyan about the need for special care in developing and in cherishing natural beauty. Unless man is now at pains to make rules for the preservation of natural beauty, consciously protecting it at the partial expense of some other activities, he would cut off his own spiritual supplies and leave his descendants a helpless prey to materialism. Dr. Bronk believes that science should be recognized for the beauty of its spiritual undertaking, and that it benefits from being carried on in a lovely setting. He finds no conflict between science and natural beauty; rather, the former makes possible the latter and the latter enriches the former.

This conception of the positive value of amenity as well as of conservation inspired much of the debate in the House of Lords which Lord Chorley opened. Lord Chorley asked quite frankly whether any part of Britain can be made safe from desecration; and in spite of the deep feeling for natural beauty of men like Lord Mills, Sir Christopher Hinton and Sir William Holford, and their efforts to defend it, that remains one of the critical questions. Lord Chorley referred with deep appreciation to Sir Christopher's address to the Royal Society of Arts last November, and Earl Waldegrave, speaking for the Government, also paid tribute to Sir Christopher's address and to that of Sir William Holford, from both of which he quoted. Lord Chorley was particularly concerned with the effect of transmission lines on the landscape and the reluctance of the electricity boards to put even sections of low-tension cables underground, urging that such decisions should not rest with the electricity authority's engineer; and he also suggested that the power to make decisions in matters of this kind should rest in independent hands not primarily concerned with power.

Neither Lord Waldegrave nor Lord St. Oswald, replying for the Government, was altogether reassuring, the former arguing that the Minister of Power's inquiry was unbiased and the latter pointing out that the Franks Committee had not recommended that inspectors who conducted planning inquiries should be independent of the Government. Both stoutly claimed their concern for the preservation of natural beauty; but it is, of course, the actual course of events that has roused this deep concern—a

concern reflected in almost every speech in the debate, particularly in that of Lord Birkett. Perhaps the most noteworthy speeches, however, were those of Lord Hurcomb, Lord Citrine and Lord Conesford.

Lord Hurcomb began by observing that the preservation of rural England and its flora and fauna could and should be one of the most effective remedies for the strains and stresses of industrial and urban life. The preservation of Nature is one of the most urgent needs of man, and is entitled to the highest priority. If we neglect it, he said, we imperil the health and sanity of future generations. Nevertheless, there is little indication that this is recognized in Government policy, and Lord Birkett later pointed to its bearing on the needs of youth. Lord Hurcomb then, from his earlier experience as chairman of the Electricity Commission, made several constructive suggestions for dealing with the difficulties of transmission. First, he maintained that those who were to benefit from the expansion of electricity supply ought to afford, and can afford, the cost of observing proper standards of amenity, just as they have to observe proper standards of safety although lower standards might be cheaper. Where it is desirable to put the transmission line underground, however, that should be accepted as a proper part of the costs of the scheme and dealt with in some way as capital expenditure. As regards public inquiries, however, Lord Hurcomb thought that usually a trained departmental inspector gives general satisfaction and is the only practicable administrative procedure; but where much controversy is involved and wide issues of public policy, it might be wise for the Minister to appoint someone unconnected with the industry or with his department.

Lord Citrine, speaking both as a lover of the countryside and from his experience as a part-time member of the Electricity Council, welcomed much that had been said in the debate. He believes that the electricity boards have not interpreted their responsibilities narrowly, and he quoted Sir Christopher Hinton as putting amenity first of all their problems and regarding transmission lines as providing the most difficult and intractable problems. Generally, he supported Lord Hurcomb's suggestions, particularly for putting selected distribution lines underground; but he also emphasized the economic aspect and pointed out that a decision must ultimately be made as to whether the consumers of electricity could fairly be expected to pay the entire increased costs incurred in safeguarding amenity.

Lord Citrine himself had referred to Sir William Holford's outstanding work, and Lord Conesford's speech was notable for quoting the principles laid down by Sir William for siting transmission lines. Sir William's basic rules are broadly to avoid altogether the major areas of highest amenity value, even if the total mileage is somewhat increased, and to avoid smaller areas of high amenity or scientific interest by deviation, where this can be done without using too many angle towers. Other things being equal, Sir William would choose the most direct line with no sharp changes of direction, and would select tree and hill backgrounds in preference to sky backgrounds, securing this opaque background as long as possible when the line has to cross a ridge, and crossing obliquely at a dip. He prefers moderately open valleys with woods; in flat and sparsely planted country he would keep the high-voltage lines so far as possible independent of smaller lines, converging routes, distribution poles, etc., so as to avoid the birdcage effect of which Lord Chorley complained in the debate. He would also approach urban areas through industrial zones where these exist; where pleasant residential and recreational land intervenes, the comparative costs of putting cables underground should be examined carefully for all but lines of the highest voltage.

Sir William believes it is well worth while to clean up many small untidy areas, and he is fully prepared to put the smaller lines underground in appropriate instances. Lord Conesford fully supported what Lord Birkett had said about the disregard for a statutory obligation by some of the electricity boards, as in a recent proposal on St. David's peninsula, Pembrokeshire, on which there was a public inquiry on January 27, 1960. He believes that, with early consultation, planning and observance of the law as it now stands, electrical development can proceed without unnecessary destruction of amenities; and it may be recalled that on November 20 the Government accepted a resolution moved by Mr. W. F. Deedes in the House of Commons urging a fresh appraisal of forthcoming demands and the stimulation by every suitable means through industry, planning authorities, professional bodies and voluntary institutions of higher standards of siting and design within the landscape of Britain. There may be occasions when it can fairly be asked whether a particular development is really essential. There are certainly others when its place in a Nature reserve or national park is open to challenge as detrimental to the nation's real interest. There can seldom be occasions when it is not expedient to bring to the task of development the highest standards of design and the soundest principles of siting.

MODERN TRENDS IN ENZYMOLOGY

Enzymes

Lectures held at the Conference on Enzymes and Their Action, Wageningen, April 6–9, 1959. Pp. 158. (Zwolle: N.V. Uitgevers-Maatschappij W. E. J. Tjeenk Willink, 1959.) n.p.

THE seven articles which are published in this volume represent the papers which were given at the Agricultural University, in Wageningen, during an international conference on "Enzymes and Their Action". The purpose of these conferences is to provide research workers in different fields of science with information about recent developments and results from a number of different areas of research. This aim has been fulfilled to a large extent during the last conference, and the result is the production of a book, which, in a small number of pages, contains an

extremely large amount of useful information, given by a number of leading authorities in the wide field of enzymology. It is a slight disappointment, however, that neighbouring fields such as cytology and genetics have been somewhat neglected, except by the last author. For this reason, the book does not clearly show the real importance of the part played by enzymology in the biological sciences as a whole.

In the introduction, E. C. Slater follows a historical approach and gives, in a table, some landmarks in the history of enzymology; he then goes into the characteristics of enzymes, their protein nature and their catalytic nature. A discussion of the mechanism of enzyme action follows, based on the kinetic, the isotope and the chemical methods. Finally, Slater emphasizes the advantages presented by enzymes as tools in other investigations, such as analytical procedures and structural determinations.

The next lecture, by C. de Duve, is devoted to the intracellular localization of enzymes by means of centrifugal methods; his report is a very thorough and useful discussion of the various methods which can be used for the separation and isolation of the cell constituents present in a homogenate. Readers who are more interested in results than in methods will be a little disappointed by the fact that lysosomes are barely mentioned by their discoverer in this article.

P. Desnuelle, in his review on the structure and the mode of action of proteolytic enzymes, had to limit himself to the proteolytic enzymes of digestion. He begins with a short, but very good, discussion of protein structure, with insulin and ribonuclease as examples; he then deals in detail with the biochemical changes which occur when enzyme precursors (pepsinogen, trypsinogen, chymotrypsinogen, for example) are transformed into active enzymes. He ends with an interesting discussion of the specificity of the proteases and the configuration of their active centre.

In the next chapter, E. C. Webb gives an accurate description of co-enzymes and prosthetic groups; the structures of flavin-adenine-dinucleotide, diphosphopyridine nucleotide, triphosphopyridine nucleotide, hydrogen carriers, lipoic acid, co-enzyme *A*, ubiquinone, uridine diphosphate are reviewed. Activation of certain enzymes by ions is also discussed.

Sir Rudolph Peters deals with the inhibitors of enzymes, especially with the effects of fluoroacetate on aconitase, of arsenicals on lipoic acid, of thiol and cholinesterase inhibitors. He emphasizes the importance of the subject not only for theoretical reasons but also for the search for antidotes.

A very complete review by F. Dickens on the regulation of enzymic reactions follows; the emphasis is placed on carbohydrate metabolism, but the study is a very thorough one and such factors as diabetes, lactation, effect of adrenal corticosteroids, etc., are incorporated in the chapter.

The book ends with a lecture by H. Chantrenne on the biosynthesis of enzymes; this lecture is unfortunately a short one, but it is also a very clear one. All the essentials about induced enzyme formation, genetic control of enzyme synthesis, general mechanisms of protein synthesis will be found there in concise form.

In conclusion, this book, despite minor shortcomings, will prove extremely useful for all those —and there are many—who are looking for an appraisal, based on a number of important examples, of the present status of enzymology. J. Brachet

GEOLOGY OF PETROLEUM

Géologie des Pétroles

Principes et Applications. Par Prof. Maurice Robert. Pp. iv+285. (Rhode-St-Genese: Editions de Visscher; Paris: Gauthier-Villars, 1959.) n.p.

THIS posthumous work of Maurice Robert, lately professor in the University of Brussels, authority on the geology of Central Africa, and distinguished oil technologist, is a new, if not strikingly original, approach to this subject. The author was unable to complete the manuscript, and the book owes much in preparation to Paul Fourmarier (University of Liège), Jean Devroey (Royal Academy of Colonial Sciences), Alfred Errera (University of Brussels), especially to Eugène Lecomte, the author's secretary. It is divided into "Principles" (occupying nearly two-thirds of the text) and "Applications", and opens with an outline of the chemistry of petroleum; thereafter treatment is to a conventional, but not elaborate, geological pattern.

Genesis of petroleum from sapropelic organic matter; fundamental characteristics, chiefly sedimentary rocks, forming parent and natural storage environments; oceanic and continental waters and their significance for oilfields, including their influence on salt-dome deposits: these are lucidly discussed. Oil migration, distinction between primary and secondary movements leading to correspondingly phased oil-deposition, also the nature of the diverse forces responsible for these phenomena, are stages portrayed in the evolution of oil-pools.

There is a condensed essay on oil-accumulation in suitable reservoirs, the existence of which depends on sediment-facies or on structural (orogenic) manifestations, or both; interposed briefly are remarks on modern technique in exploring deeply buried strata for oil, namely, geophysical methods, a subject seemingly out of place in the essentially theoretical trend of this book. The possibility of locating oil-pools in non-marine sediments is not overlooked, nor is destruction (diagenesis) of petroleum by oxidation or metamorphism, but discussion is terse and at most directive.

Surface indications of petroleum are described traditionally and the proper balance between positive and negative signs (the latter, *les faux amis*, an appropriate if somewhat cynical expression, typically French, with an assured place in 'wild cat' drilling parlance) is maintained. The sequel concludes Part 1 with dissertations on potential oilfields assessed according to fundamental geological conditions to be satisfied *a priori* in search for favourable terrain; on distribution of oilfields (geosynclinal, epirogenic environments, etc.); on current ideas on petroleum generally, with a summary of its geological and geographical distribution.

In Part 2, on the application of principles to studies of oil-deposits, examples are confined to the Middle East, North Africa, western and eastern fringes of that continent (Madagascar and some interior occurrences), with shortened versions of oil in France, Germany, Holland, Belgium and Great Britain. Within their setting, descriptions are good, and line-drawings and maps are helpful. But the whole treatment here suffers from serious restrictions, geographically, and in the number of pages allotted (76 of the total of 279). To omit at least a résumé of oilfield geology of the Americas, with the magnitude of technical data yielded by them to science, invites

unavoidable criticism. Had the author lived to complete his work, such omission would doubtless never have occurred. His realistic grasp of basic principles, so evident throughout, would have been enriched by this more spatial survey of their applications.

The book is reasonably documented, but lacks an index. The editors are commended for the discharge of a difficult task. For that reason, apart from perpetuating the name of such a brilliant geologist as the author, this volume deserves success; it should be in every library embracing the literature on petroleum.

H. B. MILNER

SOUNDING ROCKETS

Sounding Rockets

By Homer E. Newell, jun. (McGraw-Hill Series in Missiles and Space Technology.) Pp. vii+334. (London: McGraw-Hill Publishing Co., Ltd., 1959.) 97*s*.

THE use of sounding rockets for making scientific measurements in the Earth's upper atmosphere first gained world-wide attention during the International Geophysical Year, when some 200 rockets were launched by the United States alone. However, experiments of this kind began more than ten years earlier and a wide variety of research rockets and adaptations of military rockets have since been used for research purposes. Scientific conferences on the new techniques and results have gradually become widespread, and this book grew out of papers given at one such symposium organized by the American Rocket Society during November 1956. It is the work of almost a score of collaborators under the general editorship of Dr. Newell, who is now the assistant director for space sciences in the U.S. National Aeronautics and Space Administration.

Most of the book is devoted to descriptions of sounding rockets developed by France, Japan, the United Kingdom and the United States. The history of the development, and details of the propulsion system, construction features and launching methods, together with performance details are given in almost all cases. There are chapters on balloon- and aircraft-launched rockets, including a few figures of costs, and on the range facilities available for rocket firings. The book is copiously illustrated by photographs and diagrams with numerous references and a reasonable index. A final short chapter on Earth satellites is written by Dr. Newell, but does little more than list some of the experiments such vehicles make possible and give a brief account of basic orbital characteristics.

The details of the various rocket vehicles will be of value to workers engaged in the design of such devices, and much previously inaccessible information is usefully gathered together. The account of the physical properties of the high atmosphere and of the ionosphere, aurora, cosmic radiation, airglow, magnetic field, etc., given in Chapter 2 is so highly condensed that what might otherwise have been one of the most interesting parts of the book seems likely to please neither the specialist nor the newcomer to the subject. In contrast to the careful listing of firing procedures and geographical location of firings completed, the extremely brief description of the results achieved and of the apparatus used for the measurements is very disappointing, although the interested reader could no doubt find much information among the hundred or more references listed throughout the book. Despite these criticisms, the book can be recommended as a useful history of the many rockets used for upper atmosphere research during the past decade and a guide to the pay-load and performance capabilities of the various types.

W. H. STEPHENS

POISONOUS AMPHIBIANS AND REPTILES

Poisonous Amphibians and Reptiles

Recognition, and Bite Treatment. By Prof. Floyd Boys and Prof. Hobart M. Smith. Pp. xiii+149. (Springfield, Ill.: Charles C. Thomas; Oxford: Blackwell Scientific Publications, 1959.) 36*s*.

FORTUNATELY it is fairly uncommon to find present-day authors of scientific books prone to the worst style of pompous and woolly writing—'gobbledygook'—for the great majority do produce works that are both factual and concise.

Although the term 'gobbledygook' was coined in the United States, not all its citizens, evidently, have yet learned to avoid writing jargon, and Boys and Smith's book is considerably marred by its phraseology and ambiguity. One of their prime examples of obfuscation is, "If the site of the bite is such that there are no anatomical contraindications to the suggested maneuver . . .". The book is intended primarily as a practical manual for the recognition of poisonous reptiles and amphibians and for first-aid treatment of bites; but the average person, particularly in an emergency, would surely grasp more quickly the advice "call a doctor" than ". . . the best procedure is to request an immediate house call there by the physician contacted".

Much extraneous material has crept in, and some relevant matters have been omitted. An account of snake locomotion and rattles, for example, is out of context, while two frogs reputedly poisonous are not named, being merely described as red-legged, one occurring in the eastern and one in the western United States. Presumably the eastern one is *Rana palustris*, the pickerel frog, and the other is *Rana aurora*. the red-legged frog, yet examples of the first species are often yellow or orange coloured on the thigh, not red. Incidentally, the inference in the description is that only one species is involved.

The book purports to be limited in scope to those species occurring in and around North America, yet the species of 'common toad' figured is a British natterjack. Mention is also made of the extreme caution with which the South African boomslang should be handled—a good point, however, in view of the tragic death of the doyen herpetologist, Dr. K. P. Schmidt, and of the flippant manner in which many persons handle rear-fanged snakes.

In the section dealing with first aid, the use of potassium permanganate crystals is rightly decried. In earlier works, this substance was invariably recommended as suitable treatment for snake bites, but it was later found to have deleterious effects and is therefore now omitted from snake-bite kits.

The authors do not tread new ground by producing this book but instead have collated and revised previous texts on similar subjects and assembled the information as a unit.

A. G. C. GRANDISON

ASTRONOMY IN AUSTRALIA

STELLAR INTERFEROMETER OF THE SCHOOL OF PHYSICS, UNIVERSITY OF SYDNEY

THE Department of Scientific and Industrial Research of Great Britain has made two grants totalling £A.100,000 towards the intensity interferometer project in the Chatterton Astronomy Department of the School of Physics, University of Sydney.

The University of Manchester and the Chatterton Department are undertaking the project on a joint basis. The grant of £A.100,000 is to be the University of Manchester's contribution towards the cost of the project, estimated at present to be approximately £A.200,000.

An original grant of £A.6,250 from the Department of Scientific and Industrial Research was used to carry out a design study of the interferometer. The remaining £A.93,750 will largely be used to meet a portion of the constructional costs of the instrument. The School of Physics will meet the remainder of the cost of the project, which will include the development of a site and running costs.

Great interest has been expressed by astronomers in the intensity interferometer, which will be the first and only instrument of its kind in the world.

Dr. R. Q. Twiss, senior lecturer in the Chatterton Astronomy Department, and Mr. R. Hanbury Brown, at present at Jodrell Bank, the University of Manchester's radio-astronomy station, will be in charge of the project. These two scientists were responsible for the original concept of the instrument and also for its general design. Mr. Hanbury Brown will be making frequent visits to Australia to partake in the work with the new instrument.

The design of the instrument was carried out in England during 1959 under the supervision of Dr. Twiss and Mr. Hanbury Brown. A contract for the major electronic equipment associated with the interferometer has been let to Mullards (Great Britain). Dunford and Elliott of Sheffield are the consulting engineers responsible for the construction of the interferometer. It is expected that the construction of the interferometer will be completed towards the end of 1960 and that the instrument will be assembled at a field station at Narrabri, New South Wales, early in 1961. This site was chosen after considerable study of weather and other conditions at a number of centres in New South Wales.

The most important parts of the stellar intensity interferometer are the two large mirrors, some 25 ft. in diameter, shown on their movable mounts in the artist's sketch (Fig. 1). These mirrors, which, with

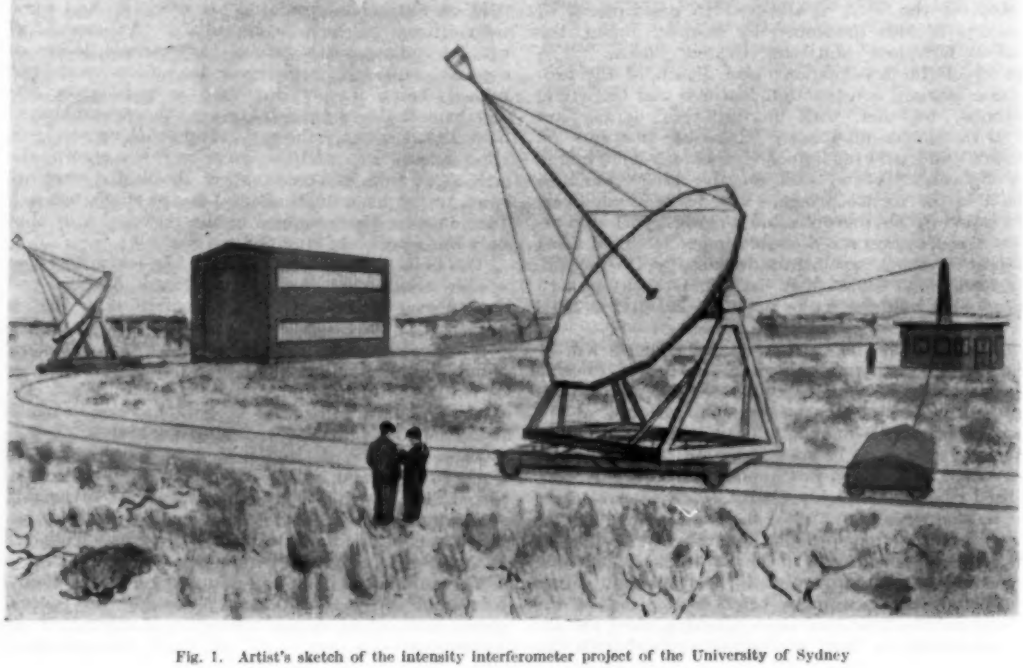

Fig. 1. Artist's sketch of the intensity interferometer project of the University of Sydney

their mounts, each weigh about 12 tons, do not have a continuous glass surface like a normal telescope mirror since this would be prohibitedly expensive. Instead they are built up of 252 identical hexagonal mirrors which focus the light from the star on to the cathode of a photomultiplier tube. There is no question of trying to image the star, so the elementary mirrors can be crude, and therefore cheap, by normal astronomical standards.

The output current of the photomultiplier tubes is linearly proportional to the intensity of the incident starlight. The fluctuations in this current are passed back to the control hut on the right-hand side of the picture by cables, suspended from catenary wires anchored at one end to a central mast and at the other end to cable trucks, connected to the movable mirror mounts. By comparing the fluctuations in the two photo currents at different spacings of the mirrors, one can obtain a direct measurement not only of the size and temperature of the star but also of the way in which its brightness varies over the stellar disk. The knowledge of these quantities is of great importance in astrophysical theory, and for the majority of the hotter stars at least it cannot be obtained directly in any other way.

The large building on the left of the picture is a garage where the mirrors are housed during the day to keep off the dust and to protect them from the direct rays of the sun. The control hut on the right-hand side of the picture contains all the electronic equipment and is where the observers work.

During an observation the mirrors move around a circular track some 600 ft. in diameter in such a way that the line joining their centres is of constant length and at right angles to the star, which appears to move across the sky due to the rotation of the Earth. The positioning of the mirrors is fully automatic and is determined by a computer in the control hut, which also points the mirrors at the star.

With this instrument, which is the first of its kind, it should be possible to measure about 140 of the brightest stars in the sky. A large fraction of these are very hot stars, the size of which is in considerable doubt, most of them being in the southern sky. Indeed, some of the most interesting stars are so far south that they cannot even be seen from England, which is one of the main reasons for setting up the interferometer in Australia.

In principle it should be possible to build an intensity interferometer with much larger mirrors and with more elaborate electronic equipment with which one could measure several thousand stars. However, such an instrument would be much more expensive and any decision as to whether it should be built would naturally depend on the results obtained with the present interferometer.

The use of an intensity interferometer is inherently limited to observations on the hotter stars. In order to make measurements on the cooler stars it is proposed to build a re-engineered version of the Michelson stellar interferometer. The detailed design of this instrument is still in the early stages, but it is hoped to develop a system, using the latest servo and auto-collimation techniques and a maximum baseline of the order of 100 ft. with which observations can be carried out on any sufficiently cool stars brighter than fifth magnitude.

METEOROLOGICAL USE OF PULSED DOPPLER RADAR

By J. R. PROBERT-JONES

Meteorological Research Unit, Royal Radar Establishment, Great Malvern

A 3-cm. pulsed Doppler radar became available at the Royal Radar Establishment for use by the Meteorological Office in October, 1958. Mr. Boyenval, of the Royal Radar Establishment, had already shown that this equipment could be used for measuring the speed of fall of precipitation particles and, under certain conditions, for obtaining their size distribution, and this article describes a few results from the programme of observations which followed.

The radar, operated at Pershore, 12 miles east of Malvern, enables the radial velocities of targets to be found from the Doppler frequency shift of the returned echo. The information is produced in discrete form and is displayed as a matrix, each row corresponding to a range interval along the beam of about 150 m., and each column to a velocity interval of $\frac{1}{2}$ or 1 m./sec. The echo power from the scatterers in each range interval and velocity band is summed and presented on the matrix as intensity modulation. The display is filmed by a 35-mm. camera, each frame having a nominal exposure of 5 sec. A picture of a typical display is shown in Fig. 1.

The radar is normally used with a vertical beam, and this gives the vertical velocities of precipitation particles. These vertical velocities are the resultant of the speed of fall of the particles with respect to the air and the vertical air motion. In warm-frontal conditions Bannon[1] has shown that the latter is small, and negligible in comparison with the velocity resolution of the radar, and below the melting band the particles will be raindrops. Thus here the observed velocity spectrum would be that of the terminal velocity of the raindrops, which, from the unique relationship between size and speed of fall, gives their size spectrum. By measuring the relative power in each element of the matrix and using the Rayleigh approximation for scattering by small spheres, the drop-size distribution can be found at all heights below the melting band.

Three examples will now be given of the information which can be derived from the radar observations.

On April 15, 1959, a depression moved quickly across the Bay of Biscay to the Cherbourg peninsula, where it remained, filling slowly. The next day, observations were taken during the passage of a rain belt which spread north across England ahead of the associated warm front. Concurrently, an aircraft of the Meteorological Research Flight from Farnborough flew over the radar at height intervals of 300 m. taking drop samples by the aluminium foil

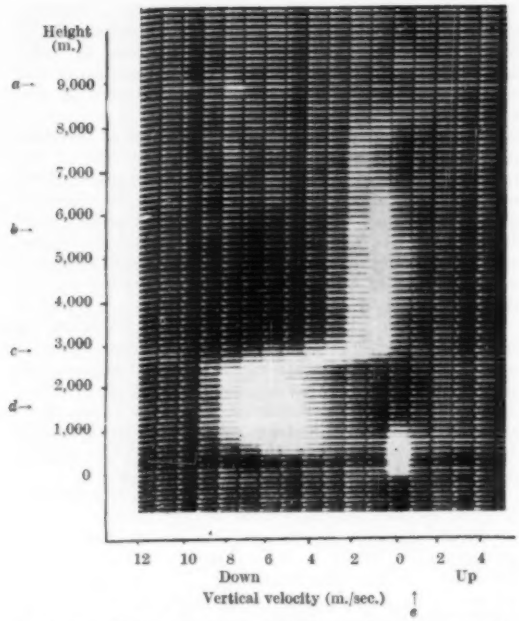

Fig. 1. Photograph of a typical display from the vertically pointing Doppler radar. *a*, range marker; *b*, echo from snow; *c*, acceleration zone as snow melts (melting band); *d*, echo from rain; *e*, transmitter pulse and ground break echo

impactor technique described by Bigg *et al.*[2] and by Garrod[3]. Seven samples were obtained between 900 m. and the base of the melting band at 1,800 m. for comparison with the radar observations.

A typical plot of the drop-size distributions obtained by the two methods is shown in Fig. 2. The radar threshold curve for concentrations below which the signal would be lost in noise is also given. The radar signal intensities are relative to noise, but absolute values have been put on the calculated concentrations by evaluating theoretically the noise intensity, and by comparing on a previous occasion calculated and observed rates of rainfall. It can be seen that no drops of radius greater than 0·75 mm. were detected on the foil, while the radar analysis indicated that drops of more than 1 mm. were present. This difference was found on every occasion and is to be expected. It can be shown on statistical grounds that an aircraft impactor which can only conveniently sample 0·1–0·2 m.^{3} of air, as in the equipment used, is not accurate in the observed rainfall-rates of 1–3 mm./hr. for drops of radius more than about 0·4 mm., and is unlikely to detect drops greater than 1 mm. To have detected the larger drops recorded by the radar in their observed concentrations the sampling volume would have had to be increased by a factor of 10^3–10^4.

Direct comparison between aircraft and radar methods was possible for 24 rain-drop concentrations at radii from 0·25 to 0·75 mm. The agreement obtained was good, the root mean square of their difference, after allowing for the observational errors of both methods, being about 20 per cent. We are encouraged, therefore, to use the radar data to obtain drop-size distributions.

Ten sets of drop-size distributions were obtained on the evening of November 1, 1958, during the passage of a rain belt associated with the eastward-moving warm fronts of a complex low near Iceland. These showed an apparent decrease by a factor of 20 in the number of 1-mm. drops between 1,200 and 800 m., and a corresponding but smaller increase in the number of small drops. There was also marked variation in the calculated distribution with height of the vertical flux of liquid water. Yet there was evidence from the radar records that a steady state existed below the melting-level. We are forced to the conclusion that the vertical air motion was not negligible on this occasion, as had been assumed.

Distributions and fluxes were therefore computed for various assumed values of vertical air velocity, and it was found that a downward air motion of 1 m./sec. at 1,500 and 2,200 m. relative to that at 800 m. is capable of eliminating completely the apparent variation of flux with height. Moreover, the recomputed distribution curves, assuming this value, no longer show more large drops and fewer small ones at the higher levels. The analysis shows that the effect lasted for at least 1½ hr., the most probable cause being an orographic standing wave due to the Malvern Hills or perhaps the Welsh mountains, which both lay directly upwind.

An entirely different use of the Doppler radar is in wind measurement. A technique has been developed for the measurement of the horizontal wind at various heights in frontal zones, from which an estimate of the field of convergence may be made. The beam can be brought down to low angles of elevation to measure the component along the beam of the velocity of the precipitation particles. They will be moving with the wind and will also have a speed of fall w. For warm front precipitation at heights above the freezing level, w will have a small spread, and its value, in the absence of appreciable vertical movement of the air, will be about 1 m./sec. Using this value for w the component of the horizontal wind towards the radar can be found. Provided there is little variation of wind in the horizontal over distances of up to 4 km., two such component measure-

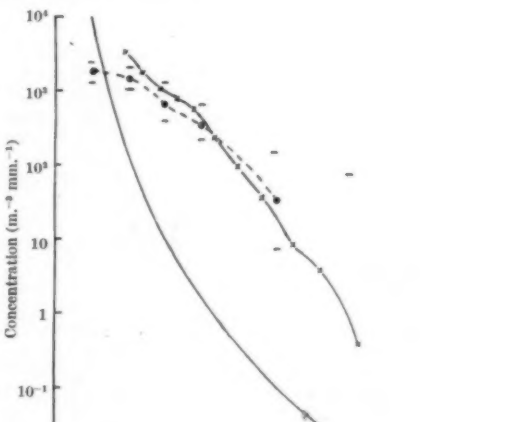

Fig. 2. Drop-size distributions, 1528, April 16, 1959, at 1,500 m. × — ×, radar concentrations; ⊙ – – – ⊙, aircraft concentrations with their upper and lower 5 per cent probability limits; ——, radar threshold curve

ments on azimuths 20° apart enable the wind vector to be found with an estimated accuracy of about 2 m./sec.

An experiment to test this theory was carried out on January 6, 1959, when a slow-moving warm front lay along the south coast, with the frontal zone over Pershore between 800 and 1,400 m. Several values for the wind were calculated at 600, 900 and 1,200 m.; the scatter in these measurements was consistent with the estimate of the accuracy given above.

The potentialities of the pulsed Doppler radar as a tool of meteorological research have by no means been fully explored, but enough has already been done to show that we have equipment capable of providing information of a type and in a quantity unapproachable by any other means, which will be of great interest both to the cloud physicist and the synoptic meteorologist.

Acknowledgment is made to the Chief Scientist, Ministry of Aviation, for making available the equipment, and to the Director-General of the Meteorological Office for permission to publish this article.

[1] Bannon, J. K., *Quart. J. Roy. Met. Soc.*, **74**, 57 (1948).
[2] Bigg, F. J., McNaughton, I. I., and Methven, T. J., R.A.E. Tech. Note Mech. Eng. 223, Min. of Supply (1956).
[3] Garrod, M. P., Air Ministry, London, Met. Res. Comm., M.R.P. No. 1050 (1957).

PRONENESS OF DRIVERS TO ROAD ACCIDENTS

By Dr. R. J. SMEED

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ROAD accidents are responsible for the deaths of about 6,500 people a year in Britain, for about 37,000 deaths in Western Europe outside Britain and for about the same number in the United States. Figures for road accidents in the World as a whole are not available, but they are known to be responsible for injury to millions annually, and these numbers are increasing. The subject of road accidents is therefore one of considerable social importance and it has become increasingly a topic of scientific investigation. One of the ways in which the subject has been tackled is in the detection of so-called 'accident-prone', that is to say, of people who, because of their physiological or psychological traits, are more prone to accidents than others.

Many of us—in our everyday life—are acquainted with drivers whom we believe to be accident prone, and, since the concept of accident proneness with regard to industrial accidents was put forward on a scientific basis by Greenwood and Yule in 1920 [1], several hundred papers have been published in the scientific journals giving the results of investigations into the proneness of drivers to road accidents. Unfortunately, few results of scientific value have emerged. Some of the reasons for this lack of success are not difficult to find. Drivers often have an incentive to conceal any accident in which they have been involved, and they often have little difficulty in doing so. Those working for commercial or State enterprises usually have more difficulty than others in concealing accidents, but here other considerations are important. If a professional driver has an apparent tendency to be accident prone the employer is likely to dismiss him or put him on to other duties. Although this may be a good policy from the point of view of reducing accidents in the particular organization involved, it makes scientific investigation of the subject difficult. But the difficulties of obtaining non-corrupt data are by no means the only ones; the statistical difficulties are real.

The number of recorded accidents for each driver per annum is usually of the order of 0·2–2 if minor damage accidents are being examined, 0·05 in the case of minor personal-injury accidents and 0·01 if attention is confined to serious accidents. Consequently chance plays a large part in the actual distribution of accidents. Thus, if one takes a group of drivers each of whom in a given period might, on the basis of past experience or some other basis, be expected to have two accidents, then because of the operation of the laws of chance, and, in consequence, the expected numbers of accidents obey the Poisson Law, a proportion e^{-2}, or 14 per cent, will have no accidents in the period under consideration. On the other hand, if one takes a group of drivers who in this period would be expected to have 0·5 accidents each, clearly less liable to accident than the others, 39 per cent of them will have at least one accident (since $1-e^{-0\cdot5}=0\cdot39$) and 9 per cent of them two or more. It is therefore, in practice, not possible to identify, with certainty, the accident-prone individuals on the basis of the accidents they sustain.

There are also other difficulties. Before one can prove that the members of a particular group of drivers are, because of their personal qualities, more prone to accident than another group of drivers, one must be confident that the two groups of drivers drive under similar conditions, since it is known that the frequency of accidents for unit distance travelled is different on different types of road, at different times of the day and at different parts of the year.

A high proportion of the investigators of accident proneness have not fully realized the difficulties of their tasks. This, fortunately, cannot be said of the book by Sauli Häkkinen, which discusses the concept of accident proneness, reviews much of the previous work on the subject, and describes the investigations made by the author, with the help of the members of the staff of the Institute of Occupational Health in Helsinki*. The investigator analysed the accidents occurring to the drivers of buses and trams of the municipality of Helsinki and correlated the results of psychological tests on samples of drivers with their accident experience. Like some previous investigators, he divided the factors affecting accident proneness into: (1) factors which remain constant over the period of exposure; (2) factors which change slowly with time, especially age and experience;

* "Traffic Accidents and Driver Characteristics: a Statistical and Psychological Study." (Finland's Institute of Technology—Scientific Researches, No. 13.) Pp. 198. (Helsinki: Finland's Institute of Technology, 1958. Obtainable from Akateeminen Kirjakauppa, Helsinki.) 20*s.*; 3 dollars.

(3) rapidly or irregularly changing factors. The main analysis is concerned with (1).

The analysis of the effect of age and experience does not suffer from the difficulties mentioned above, and in the book already referred to, Häkkinen quotes results he obtained in a preliminary analysis of the data although the details are not given. He claims to have found a continuous reduction in the number of accidents during the first 4–5 years of employment, the number of accidents during the first year being nearly three times as high as in the fifth. He also states that there was a continuous decline in the number of accidents per annum up to the age of 45–48 years. Thirty-year-old drivers had, irrespective of driving experience, twice as many accidents as forty-eight-year-old drivers. He concludes that only if the group studied is homogeneous with respect to age and experience is it possible to isolate the effects of the constant factors on accident proneness. He then proceeds to attempt this. He analyses the distribution of total accidents occurring to various samples of drivers, and shows conclusively that the distribution occurring could not have occurred by chance from a population of drivers equally liable to accident. Some drivers were more liable to accident than others. Similar conclusions have been obtained by a large number of previous investigators. The kernel of the problem is, however, the exclusion of the possibility that the differences in the accident rates were due to exposure or other non-constant personal differences. This part of the investigation will therefore now be discussed.

Häkkinen states that during the time period under consideration most of the drivers had been operating on many different routes and that this is bound to cancel out part of the effects of the possible non-homogeneity of the routes. He nevertheless examined the accident rates on the different routes and found that they varied between 0·7 and 2·7 accidents per driver per annum. However, he states 86·5 per cent of the driving was on routes where the accident rates per driver varied between 1·1 and 1·7, and the influence of the differences between the routes could not, therefore, have been decisive. This is, however, an opinion on a topic in which exact analysis is required. Häkkinen then states that in order to clear up the matter, he computed an expected number of accidents for each driver by adding the products of the time the driver had been operating on each route and the known accident rates of these routes in a manner previously attempted by Whitfield[3]. He then divided the actual number of accidents by the expected number of accidents and correlated the resulting ratios for two successive periods. Unfortunately, Häkkinen does not give the detailed results of this most useful analysis. He merely states that the resulting correlations were of the same order as the original ones and did not differ significantly from them. He goes on to say that this is best seen from the correlation between directly evaluated accident rates and those calculated by adjusting the accident rates per driver by the accident rates of the various routes on which they drive. He then obtained correlations of the order 0·95. The correlation is high, and Häkkinen therefore concluded that the differences between the routes could not have affected the results. I think that the argument is plausible, but that a more satisfying analysis is desirable. It is a pity that, in a book of 198 pages, more of the original data were not given so as to enable the reader to carry out some analyses himself.

Häkkinen concludes that some drivers consistently have more accidents than others when driving under similar conditions. He does not, however, discuss in detail what proportion of drivers have more accidents nor how many more accidents they have. He concludes that the correlation coefficient between the number of accidents sustained by a driver in two successive time periods is of the order 0·25–0·35 in two successive yearly periods and of the order 0·60–0·70 in successive 4-year periods. These correlations are very high. Their practical significance can perhaps be illustrated by an example. If a group of drivers can be divided into two sub-groups and one sub-group has an accident rate $(1 + q)$ times the other, it can be shown that if the product moment coefficient of correlation between the accidents in which individual drivers are involved in two successive periods is R, and the average number of accidents per driver in the group as a whole is $\bar{x}$ in each of two periods, then $\frac{R}{(1-R)\bar{x}} = \frac{p(1-p)q^2}{(pq+1)^2}$, where p is the proportion of drivers in the higher accident rate group. If the value of the left-hand side of this equation is denoted by y, it can be shown that the minimum possible value of q is $2y + \sqrt{4y(1+y)}$. In the cases in which Häkkinen obtains a correlation coefficient of about 0·6, the value of $\bar{x}$ is about 4. Putting these values into the formula, the minimum possible value is calculated to be 2·19. Thus, some Helsinki bus and tram drivers have an accident rate more than three times that of others. If, therefore, Häkkinen's results are correct, they seem to be of considerable practical importance.

Häkkinen then reviews the literature describing experiments made to find correlations between various psychological and medical tests and accident rates, and describes the battery of tests he used on a sample of 100 bus- and tram-drivers and gives the results of various calculations correlating test scores with accident rates and with one another. The tests, mostly selected on the basis of previous studies by other investigators, included ones for intelligence, mechanical aptitude, simulated driving, personality, simple and choice reaction time, and also a rating on behaviour and some attitudes.

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In eye–hand co-ordination he found that the accident group did, as a rule, worse than the safe group, the difference between the averages being significant. Also several variables were discovered in the choice reaction tests (which included distraction) which significantly distinguished the accident group from the safe one. Some psychomotor personality tests gave significant differences for the two groups of drivers, but the 'personality inventory' tests gave no significant differences. He claims that he may have discovered some psychological factors, particularly those of 'motor hastiness and distractability', which give rise to accident proneness and which have not been ascertained with equal clarity in previous similar studies.

Some of the methods used in the analysis are, however, not satisfactory. Häkkinen well realizes that many of his results would be upset if there were material differences in age between the various groups of drivers with high and low rate of accident. Instead,

however, of discovering the likely effects of the age differences that exist on the accident rates and test scores, Häkkinen calculated the statistical significance of the differences between the mean ages of the various groups of drivers. But this is irrelevant. If all the low-accident group were born on one day and the high-accident group one week later, the differences in mean age calculated would have been statistically significant but the effect on the analysis nil. If, however, two groups of 4 drivers were taken, one group consisting of 3 drivers aged 30 and 1 aged 45, while the other group consisted of 1 driver aged 30 and 3 aged 45, the differences in age—as calculated—would not have been statistically significant, but the effect on the analysis great. The fact that the analysis is clearly not satisfactory in one respect leads one to doubt it in others. For example, the report gives means and correlation coefficients and results of tests on their significance but, since results for individual persons are not given, it is not possible to check that the tests have been carried out correctly and used in conditions where they apply, that is to say, when the correlation is not due to one or two outlying individual points. The author does, however, make it clear that he realizes some of these difficulties.

There can be little doubt that Häkkinen has carried out one of the most important investigations on accident proneness yet made. It would, however, be useful to re-examine his results.

[1] *J. Roy. Stat. Soc.*, **83**, Part 2 (March 1920).
[2] Whitfield, J. W., "Unit of Applied Psychology" (Report No. 71, 1947).

THE NUCLEOLUS PROBLEM

By Dr. J. L. SIRLIN*
Institute of Animal Genetics, University of Edinburgh

IT is probably true to say that of the major cell components the nucleolus is the one the function of which we know least about, despite the fact that its importance has been generally recognized since the early times of cytology. About the only point of which we are reasonably certain is that the nucleolus has a high ribonucleic acid turn-over, and this was already implied by Caspersson[1] in 1941. (Brachet suggested that another role of the nucleolus is to synthesize coenzymes[2].) Fortunately, on current ideas on protein synthesis, as indeed in Caspersson's original prediction, this turns out to be a relevant point, although it will be appreciated in what follows that we can but guess at the particular implications. This is of course one aspect in the more general problem of the function of ribonucleic acid.

For the present purpose, both the questions of the origin and of the behaviour of the nucleolus will be jointly discussed. The evidence available derives from microscopy, cytochemistry and (in the last decade or so) electron microscopy, autoradiography and chemistry. The physical aspects of nucleoli (that is, structure, fusion, etc.) are well known to be extremely plastic, and, being therefore likely to confuse rather than to clarify our ideas in their present state, they are best not considered at this time. The available evidence will be considered to lead to two interpretations and these will be used as a framework for discussion. Interpretation 1 is that the nucleolus represents one genetic locus (or very few close loci) the product of which is the nucleolar ribonucleic acid; the nucleolus would thus resemble other single chromosomal regions rich in ribonucleic acid, such as certain bands, puffs and Balbiani rings, for which a similar assumption is currently accepted. Interpretation 2 is that the nucleolus, apart from consisting of nucleolar ribonucleic acid as under interpretation 1, is also largely an assembly point for different ribonucleic acids assumed to originate at different genetic loci. Thus, interpretation 2 is more comprehensive and includes interpretation 1.

So far as the assembly of non-nucleolar ribonucleic acids in the nucleolus is concerned, interpretation 2 can be supported by indirect evidence for the collection of nuclear ribonucleic acid in the preformed nucleolus[1,3–6]. This work, in which (except for Caspersson's[1]) autoradiography is used, cannot be discussed here in detail, but it may be said that it does not prove conclusively that this collection exists. Also, by staining, Colombo[7] concluded that there is confluence of nuclear ribonucleic acid into the nucleolus. Other evidence, of a rather morphological nature, is as follows. Many cytologists, among them Rattenbury and Serra[8], Tandler[9] (who list earlier work), Karasaki[10] and others[11], observed in the nucleus what they interpreted as prenucleolar bodies deriving in different measure from the chromosomes and coalescing to form the nucleolus. In particular, Lafontaine[12] presented what appears to be convincing evidence with the electron microscope. It may be thought that chromosomal ribonucleic acids gather in the nucleolus by means of these prenucleolar bodies; but the chemical nature of these bodies has been generally not characterized, except in two cases[10,11] where nucleic acid was apparently absent. Support for interpretation 2 may come also from the observation that genes in many chromosomes (including non-nucleolar chromosomes) have an effect on the ribonucleoprotein composition of the nucleolus[13,14]; but this can be only an effect on nucleolar ribonucleic acid and not the result of chromosomal ribonucleic acids collecting in the nucleolus.

For interpretation 1 some observations by Swift[15] are relevant. His observations differed from those previously mentioned of Lafontaine[12] in the same material, and indicated that certain (probably lipid-staining) properties differ in the so-called prenucleolar bodies and the nucleolus, although both contain ribonucleic acid. Swift considered these bodies as being spindle remnants. (On the whole he introduced a salutary word of caution in the interpretation of these electron microscope images.) As such these bodies would not be thought to be a vehicle for chromosomal ribonucleic acid moving to the nucleolus. The possibility of this movement of chromosomal ribonucleic acid to the nucleolus is not mentioned in Swift's papers, although he undoubtedly realizes it. Pelling[16] considered that nucleolar ribonucleic acid in the salivary gland of *Chironomus* is synthe-

* Research worker of the British Empire Cancer Campaign.

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IT is probably true to say that of the major cell components the nucleolus is the one the function of which we know least about, despite the fact that its importance has been generally recognized since the early times of cytology. About the only point of which we are reasonably certain is that the nucleolus has a high ribonucleic acid turn-over, and this was already implied by Caspersson[1] in 1941. (Brachet suggested that another role of the nucleolus is to synthesize coenzymes[2].) Fortunately, on current ideas on protein synthesis, as indeed in Caspersson's original prediction, this turns out to be a relevant point, although it will be appreciated in what follows that we can but guess at the particular implications. This is of course one aspect in the more general problem of the function of ribonucleic acid.

For the present purpose, both the questions of the origin and of the behaviour of the nucleolus will be jointly discussed. The evidence available derives from microscopy, cytochemistry and (in the last decade or so) electron microscopy, autoradiography and chemistry. The physical aspects of nucleoli (that is, structure, fusion, etc.) are well known to be extremely plastic, and, being therefore likely to confuse rather than to clarify our ideas in their present state, they are best not considered at this time. The available evidence will be considered to lead to two interpretations and these will be used as a framework for discussion. Interpretation 1 is that the nucleolus represents one genetic locus (or very few close loci) the product of which is the nucleolar ribonucleic acid; the nucleolus would thus resemble other single chromosomal regions rich in ribonucleic acid, such as certain bands, puffs and Balbiani rings, for which a similar assumption is currently accepted. Interpretation 2 is that the nucleolus, apart from consisting of nucleolar ribonucleic acid as under interpretation 1, is also largely an assembly point for different ribonucleic acids assumed to originate at different genetic loci. Thus, interpretation 2 is more comprehensive and includes interpretation 1.

So far as the assembly of non-nucleolar ribonucleic acids in the nucleolus is concerned, interpretation 2 can be supported by indirect evidence for the collection of nuclear ribonucleic acid in the preformed nucleolus[1,3–6]. This work, in which (except for Caspersson's[1]) autoradiography is used, cannot be discussed here in detail, but it may be said that it does not prove conclusively that this collection exists. Also, by staining, Colombo[7] concluded that there is confluence of nuclear ribonucleic acid into the nucleolus. Other evidence, of a rather morphological nature, is as follows. Many cytologists, among them Rattenbury and Serra[8], Tandler[9] (who list earlier work), Karasaki[10] and others[11], observed in the nucleus what they interpreted as prenucleolar bodies deriving in different measure from the chromosomes and coalescing to form the nucleolus. In particular, Lafontaine[12] presented what appears to be convincing evidence with the electron microscope. It may be thought that chromosomal ribonucleic acids gather in the nucleolus by means of these prenucleolar bodies; but the chemical nature of these bodies has been generally not characterized, except in two cases[10,11] where nucleic acid was apparently absent. Support for interpretation 2 may come also from the observation that genes in many chromosomes (including non-nucleolar chromosomes) have an effect on the ribonucleoprotein composition of the nucleolus[13,14]; but this can be only an effect on nucleolar ribonucleic acid and not the result of chromosomal ribonucleic acids collecting in the nucleolus.

For interpretation 1 some observations by Swift[15] are relevant. His observations differed from those previously mentioned of Lafontaine[12] in the same material, and indicated that certain (probably lipid-staining) properties differ in the so-called prenucleolar bodies and the nucleolus, although both contain ribonucleic acid. Swift considered these bodies as being spindle remnants. (On the whole he introduced a salutary word of caution in the interpretation of these electron microscope images.) As such these bodies would not be thought to be a vehicle for chromosomal ribonucleic acid moving to the nucleolus. The possibility of this movement of chromosomal ribonucleic acid to the nucleolus is not mentioned in Swift's papers, although he undoubtedly realizes it. Pelling[16] considered that nucleolar ribonucleic acid in the salivary gland of *Chironomus* is synthe-

* Research worker of the British Empire Cancer Campaign.

sized *in situ*, but he did not discuss the ultimate origin of this acid. (For similar evidence in another chironomid Sirlin[17] argued that it was compatible with interpretation 2.) Partial support for interpretation 1 may be found, for reasons which will later become apparent when discussing nucleolar ribonucleic acids, in the observation of Perry and Errera[18] that the incorporation of label in a fraction of nuclear ribonucleic acid is affected by nucleolar irradiation. (The significance in this context of two further observations is difficult to assess. These are that a radiation-sensitive nucleolar fraction affects mitosis[19] and that in some cells deoxyribonucleic acid is first replicated near the nucleolus[20].) Also, Vincent[21] noted that there are probably only two proteins in the nucleolus of the starfish oocyte, one in the ribonucleoprotein and another in the remaining bulk of protein; and he concluded that probably the bulk of protein is produced by a single gene.

The fact that in various organisms the occasional absence of the nucleolar organizer is accompanied by the presence of numerous ribonucleic acid-containing nucleoli smaller than normal in many parts of the nucleus[22,23] may be used to support either interpretation. For example, in favour of interpretation 2 it may be considered[23] that, given the nucleolar organizers were present, these many small nucleoli would have coalesced into the main nucleolus or nucleoli (depending on cell type), that is, they could be equivalent to the prenucleolar bodies proposed by some authors[9–12] and thought of as conveying chromosomal ribonucleic acid to the nucleolus when the nucleolus organizer is present. Swift[15], on the other hand, claimed that these are quite supernumerary nucleoli, being produced as a consequence of the withdrawal of the competition for common precursors normally exerted by the organizer, that is, they would not normally contribute to the nucleolus. (Lin[14] found that additional nucleolar organizers in certain circumstances increase the amount of ribonucleic acid in the nucleolus.) By extending these considerations, the interesting observation that the amount of these bodies is correlated with the amount of nucleolus in the different tissues[22] can be in line with either interpretation. A further neutral argument is the apparent supporting role of the nucleolus to the cell machinery of syntheses as indicated by the appearance of the nucleolus in the embryo at the time (early gastrulation) when the bulk of specific syntheses start, without however, as shown by the above-mentioned enucleolate organisms, being indispensable for a good deal of primary histogenesis. The well-known hypertrophy of nucleoli in actively synthesizing cells and the surcharge of ribonucleic acid in the nucleolus and chromosomes in cells of embryos with arrested development (lethal hybridization, inhibition of phosphorylations) also belong in this category.

It is worth noting how the two working interpretations fit into a picture in terms of ribonucleic acid, even if indulging in some unwarranted speculation. For this a coding ratio between base positions in template deoxyribonucleic acid and in ribonucleic acid near one-to-one is assumed because, although ratios of this type are at the moment scarcely more than hypothetical, this one seems the most likely[24]. If the nucleolus represents one genetic locus (interpretation 1), it is assumed it makes only a large ribonucleic acid molecule which can code for the entire amino-acid sequence of a protein, and/or several smaller ribonucleic acid molecules. The alternative implicit here is: accepting the number of constituent deoxyribonucleotide pairs in a cistron as being generally of the order of a few thousands[25], and considering the approximate (single) chain length of known ribonucleic acids as 30–120 nucleotides for soluble ribonucleic acid[26] and 5,000 nucleotides for microsomal ribonucleic acid (with smaller sub-units) and nuclear ribonucleic acid[27], a cistron (a genetic locus) seems capable at first approximation of coding for one (or a few) large ribonucleic acid species and/or for several small ribonucleic acid species, which can be a breakdown product of the large species. The first type of ribonucleic acids could be 'raw' acid[28], which would be further elaborated by genes or by the cytoplasm to be made specific for these loci and act then as a template for protein synthesis. (This implies that specific ribonucleic acid retains unspecific sequences in its structure.) Alternatively, it could be a 'finished-off' ribonucleic acid of a species-specific (but not cell type-specific) nature, which could be concerned with the production of a rather general protein such as microsomal protein. The possibility of the existence of either of these two nucleolar-specific ribonucleic acids under interpretation 1 is inferred from the fact that the nucleolar region in the chromosome is the same in all cells of an organism and it may differ between different species[29]. Both these ribonucleic acids conform with the general evidence. The second type of ribonucleic acids (the smaller molecule) could be the transfer soluble ribonucleic acids discussed below. In addition, the nucleolus may perform unitary chemical steps, such as terminal addition of nucleotides to soluble ribonucleic acid, phosphorylation of nucleosides, etc.

If, apart from consisting of nucleolar ribonucleic acid, the nucleolus represents the site of assembly of different chromosomal ribonucleic acids (interpretation 2) gathered during or after formation of the nucleolus, then in addition to the possible activities under interpretation 1 the nucleolus may be assumed to serve as an amplifying machinery[3] for the genes in the production of gene-specific ribonucleic acids acting as templates for cytoplasmic syntheses. Recently it has been thought[30] that the nucleolus uses its own ribonucleic acid to produce microsomal protein (see preceding paragraph) which is coupled to chromosomal ribonucleic acids collecting in the nucleolus. This is an attractive possibility which must, however, be viewed against the further possibility that protein turns over in the nucleolus of growing cells but, although this is not definite at the moment, apparently less in the nucleolus of mature cells (references in Sirlin[31]). It is supported by the apparent singleness of the bulk of protein in the one type of nucleolus investigated[21] and in various microsomal proteins (see later).

Soluble ribonucleic acid and its terminal binding of nucleotides occur in the nucleus[32] and in the cytoplasm[16], and apparently at roughly similar concentrations[33]. Their actual occurrence in the nucleolus shown by Vincent and Baltus[33] seems then unlikely to be relevant to the question of information transfer to cytoplasm, and what is known of the function of this soluble ribonucleic acid (see later) points in the first place in this direction. For this question, therefore, the dilemma in terms of ribonucleic acid in these interpretations is the presence in the nucleolus of nucleolar-specific versus genome-specific ribonucleic acid.

What are then the next steps? It is increasingly being felt that investigation of the relation of nucleolar ribonucleic acids to other cell ribonucleic

acids should be rewarding, which is in fact a corollary from Crick's 'central dogma'[24], namely, that information on amino-acid sequences for protein synthesis is passed at the nucleic acid-level and not at the protein-level. (Comparison of properties of microsomal protein from various sources likewise suggests that it is rather general and mainly structural[25].) It may be an over-simplification, but at the moment is conveniently economical in hypotheses. The basic information on ribonucleic acids, including nucleolar ribonucleic acids, is as follows. Two relatively well-defined types of ribonucleic acid known at present are a low-molecular weight ($1–4 \times 10^4$) soluble ribonucleic acid (*S*-ribonucleic acid)[26], possibly specific for each amino-acid, and a high-molecular weight (*c.* $1{\cdot}7 \times 10^6$) microsomal or ribosomal ribonucleic acid (*Ms*-ribonucleic acid)[27], presumably a highly specific template for protein synthesis. (Free ribonucleoprotein particles may be related to *Ms*-ribonucleic acid[26].) *S*-ribonucleic acid binds amino-acids through suitable terminal nucleotide groups, and transfers them to *Ms*-ribonucleic acid, which is believed to polymerize them into protein. *S*-ribonucleic acid is ubiquitous in the cell and may originate in *Ms*-ribonucleic acid[26]. *Ms*-ribonucleic acid is assumed to be at least partly of nuclear origin. There is molecular flow between these ribonucleic acids[27], but its significance is as yet uncertain. In the nucleolus Vincent and Baltus[28] recently characterized a fraction as *S*-ribonucleic acid. (This was recently characterized for the isolated nucleus[32].)

Some years before, Vincent[2] described a structural ribonucleic acid of the nucleolus, probably independent of nucleolar *S*-ribonucleic acid, more abundant and with slower turn-over (phosphorus). Also Taylor and Woods[4] found 'fast' and 'slow' nucleolar and chromosomal ribonucleic acids (adenine), Swift[15] described two different ribonucleic acid-containing areas in the nucleolus, and Love and Liles[36] observed two nucleolar ribonucleoproteins. In so far as any of them could be template ribonucleic acid, the interrelation (that is, flow of polyribonucleotides) between the structural ribonucleic acid of the nucleolus, a certain fraction of chromosomal ribonucleic acid, and *Ms*-ribonucleic acid is in obvious need of elucidation. Sirlin and Jacob[5] indicated that the peripheral ribonucleic acid of the nucleolus (that is, that in common with the chromosomes and the nucleolar organizer) appears to have a greater rate of labelling (orotic acid) than the internal ribonucleic acid, and would on this basis at least partly correspond with the nucleolar *S*-ribonucleic acid (which at the time was not yet definitely characterized[32]) retained by the fixative. Later it was found that in the nucleus of chironomids, where the most intimate association of nucleolus and chromosomes is at the centre of the nucleolus, it is the nucleolar ribonucleic acid at this site which resembles in its rate of labelling (uridine) the peripheral ribonucleic acid of the nucleolus in the other cell types[16,17]. In the chironomid nucleus this ribonucleic acid label moves from the centre to the remainder of the nucleolus. In this nucleus the nucleolar region of the chromosome bearing the nucleolus is a single hypertrophied band or interband[29], and the present evidence suggests that the uptake of uridine by this ribonucleic acid at the centre of the nucleolus is connected with some function discharged by this band or interband. On current ideas on the organization of polytene chromosomes any of these regions represents one or very few genetic loci. The relations of this ribonucleic acid labelled at the centre of the nucleolus to the whole nucleolar ribonucleic acid, particularly in terms of the known fractions, and to chromosomal ribonucleic acids remain to be established.

It was my intention to indicate: First, some salient points at issue in the nucleolus problem as concerns the genetic (nuclear) control of cytoplasmic syntheses. Second, that answers to some of these points can be forthcoming through the distinction in the nucleolus of different ribonucleic acid fractions characterizable by their topography and chemistry.

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[1] Caspersson, T., *Naturwiss.*, **29**, 33 (1941).

[2] Brachet, J., "Biochemical Cytology" (Academic Press, New York, 1957).

[3] Vincent, W. S., "The Beginnings of Embryonic Development", ed. Tyler, A., Borstel, R. C. von, and Metz, C. B., 1 (Amer. Assoc. Adv. Sci., Washington, 1957).

[4] Taylor, J. H., and Woods, P. S., "Subcellular Particles", ed. Hayashi, T., 172 (Ronald Press Co., New York, 1959).

[5] Sirlin, J. L., and Jacob, J., *Exp. Cell. Res.* (in the press).

[6] Goldstein, L., and Micou, J., *J. Biophys. Biochem. Cytol.*, **6**, 301 (1959).

[7] Colombo, G., *Rend. Acad. Naz. Lincei* (8), **6**, 370 (1949).

[8] Rattenbury, J. A., and Serra, J. A., *Portug. Acta Biol.*, A, **3**, 239 (1952).

[9] Tandler, C. J., *Exp. Cell. Res.*, **17**, 560 (1959).

[10] Karasaki, S., *Embryologia*, **4**, 273 (1959).

[11] Das, N. M., and Alfert, M., *Anat. Record*, **134**, 548 (1959). Kleinfeld, R. G., and von Haam, E., *J. Biophys. Biochem. Cytol.*, **6**, 393 (1959).

[12] Lafontaine, J. G., *J. Biophys. Biochem. Cytol.*, **4**, 777 (1958).

[13] Schultz, J., Caspersson, T., and Aquilonius, L., *Proc. U.S. Nat. Acad. Sci.*, **26**, 515 (1940).

[14] Lin, M., *Chromosoma*, **7**, 340 (1955).

[15] Swift, H., "Symposium on Molecular Biology", ed. Zirkle, R. E., 266 (Univ. of Chicago Press, 1959); *Brookhaven Symp. Biol.*, **12**, 134 (1959).

[16] Pelling, C., *Nature*, **184**, 655 (1959).

[17] Sirlin, J. L., *Exp. Cell. Res.*, **19**, 177 (1960).

[18] Perry, R. P., and Errera, M., "The Cell Nucleus", ed. Holmes, B. (Butterworths Sci. Pub., London, in the press).

[19] Gaulden, M. E., and Perry, R. P., *Proc. U.S. Nat. Acad. Sci.*, **44**, 553 (1958).

[20] Harris, H., *Biochem. J.*, **73**, 362 (1959).

[21] Vincent, W. S., *Anat. Rec.*, **133**, 346 (1959).

[22] McClintock, B., *Z. Zellforschung u. Mikr. Anatomie*, **21**, 294 (1934). Beermann, W., "Biological Organization", ed. Waddington, C. H., 96 (Pergamon Press, London, 1959).

[23] Elsdale, T. R., Fischberg, M., and Smith, S., *Exp. Cell. Res.*, **14**, 642 (1958).

[24] Rich, A., *Ann. N.Y. Acad. Sci.*, **81**, 709 (1959).

[25] Beadle, G. W., "The Chemical Basis of Heredity", ed. McElroy, W. D., and Glass, B., 3 (Johns Hopkins Press, Baltimore, 1957). Pontecorvo, G., *Symp. Soc. Exp. Biol.*, **12**, 1 (1958).

[26] Hoagland, M. B., Zamecnik, P. C., and Stephenson, M. L., "A Symposium on Molecular Biology", ed. Zirkle, R. E., 105 (Univ. of Chicago Press, Chicago, 1959).

[27] Bonner, J., *Amer. J. Bot.*, **46**, 58 (1959). Cheng, P., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1557 (1959).

[28] Sirlin, J. L., and Elsdale, T. R., *Exp. Cell. Res.*, **18**, 268 (1959).

[29] Beermann, W., "Developmental Cytology", ed. Rudnick, D., 83 (Ronald Press Co., New York, 1959).

[30] Brenner, S., "Biological Organization", ed. Waddington, C. H., 98 (Pergamon Press, London, 1959).

[31] Sirlin, J. L., "The Cell Nucleus", ed. Holmes, B. (Butterworths Sci. Pub., London, in the press).

[32] Allfrey, V. G., "The Cell Nucleus", ed. Holmes, B. (Butterworths Sci. Pub., London, in the press). Hopkins, J. W., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1461 (1959).

[33] Vincent, W. S., and Baltus, E., "The Cell Nucleus", ed. Holmes, B. (Butterworths Sci. Pub., London, in the press).

[34] Crick, F. H. C., *Symp. Soc. Exp. Biol.*, **12**, 138 (1958).

[35] Dintzis, H. M., Bersoek, H., and Vinograd, J., "Microsomal Particles and Protein Synthesis", ed. Roberts, R. B., 95 (Pergamon Press, New York, 1958). Ts'o, P. O. P., Bonner, J., and Dintzis, H. M., *Arch. Biochem. Biophys.*, **76**, 225 (1958).

[36] Siekevitz, P., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **5**, 1 (1959).

[37] von der Decken, A., and Hultin, T., *Exp. Cell. Res.*, **15**, 254 (1958). Hotta, Y., and Osawa, S., *Biochim. Biophys. Acta*, **28**, 642 (1958). Bosch, L., Bloemendal, H., and Sluyser, M., *ibid.*, **34**, 272 (1959). Hecht, L. I., Stephenson, M. L., and Zamecnik, P. C., *Proc. U.S. Nat. Acad. Sci.*, **45**, 505 (1959).

[38] Love, R., and Liles, R. H., *J. Histochem. Cytochem.*, **7**, 164 (1959).

OBITUARY

Dr. Abraham Flexner

Abraham Flexner was born in Louisville, Kentucky, on November 13, 1866, and died in Falls Church, Virginia, on September 21, 1959. Though not a physician, he played a leading part in the reform of American medical education which began early in this century; though not a scholar in the professional sense, he designed, created and was the first director of an institution for non-professional study of the most advanced type.

His parents were Hebrew immigrants from western Europe, pious, intelligent, courageous and uncompromising in the conduct of their home and the training of their children. Abraham was the sixth of seven sons. His eldest brother, Jacob, became the chief support of the family after financial disaster to their father in the panic of 1873. Other brothers were Simon, who became the first director of the Rockefeller Institute, and Bernard, who gained distinction in law and literature. Their mother survived her husband by many years. Long after her death, Abraham wrote: "None of her children equalled her in respect to native endowment".

In his home, in the schools and the public library of Louisville, Flexner read many of the great classics of literature and history: one of his teachers inspired him with an enthusiasm for the study of Greek, which during his later life was "a flowing spring of interest".

In 1884, financed by his brother Jacob, Abraham entered the Johns Hopkins University, which had been founded eight years earlier by Daniel Coit Gilman. He chose the classical group of studies, worked hard and obtained his bachelor's degree in two years. "Research was in the air we breathed". "President Gilman's wisdom and courage cannot be matched in the history of American education". "Those who know something of my work long after Gilman's day . . . will recognize Gilman's influence in all I have done or tried to do". Those sentences, quoted from his autobiography, reveal the indelible impress which Gilman and the Johns Hopkins made upon him.

For four years after returning to his mother's home he helped to increase the family income by teaching in the Louisville High School; then, more through chance than design, he started a school of his own, which during the fifteen years of its existence prospered and became widely known for its excellence. President Eliot, for example, asked, "Why is it that your boys come to our colleges younger and graduate in a shorter time than boys from our more famous schools?"

Flexner's discovery of his own talents as a teacher, supplementing the influence of President Gilman and the Hopkins training, brought into being the passion for a career in education which was to direct the rest of his life. In its broader development, another factor was influential, the courage and foresight of his wife.

In 1898, Flexner married Anne Crawford, a highly gifted woman whose talents supplemented his. After fourteen years of the operation of her husband's school, she raised the question whether he was to remain content as a school teacher in Louisville for the rest of his life. The answer was "No". They decided to close the school, to go to Cambridge for a year of graduate work at Harvard and then to spend two years, mainly in association with German scholars and in study of German educational organizations and methods. They made no further plans; this was their "leap in the dark".

During the third of these years, 1908, Flexner wrote his first book, "The American College", in which he analysed defects in the American collegiate system which he believed to be responsible for a student's loss of enthusiasm for scholarship during his college career.

The book came to the attention of the president of the Carnegie Foundation for the Advancement of Teaching, Henry S. Pritchett. Its impact led Mr. Pritchett to persuade Flexner to conduct an educational study of medical schools in the United States and Canada. Supported also by the Council on Medical Education of the American Medical Association, the work was begun in December 1908 and the report published in 1910 as Bulletin No. 4 of the Carnegie Foundation. In histories of medical education in America it has justly been called "the beginning of a new era", the "Magna Charta of American medical education".

The survey was made from the point of view of an educator, not a practitioner. Each of the 155 schools then existing in the United States and Canada was visited and evaluated in terms of entrance requirements, size and training of faculty, adequacy of laboratory and hospital facilities and amount and disposition of financial support. In Part 2 of the report each school was named, the verified facts in the above categories were stated, and a commentary, always frank and in many cases scathing, appended concerning faults and virtues.

Only some twenty schools were found to require two or more years of college for entrance; fifty others demanded four years of high school or 'equivalent', and many of these used that word as a way of evasion; the rest, grouped as basely mercenary, required little more than the rudiments or recollections of a common school education. Flexner's criticisms of these were fearless and uninhibited; conditions were described as "sordid, hideous, unintelligent even when honest" . . . commercial schools characterized as "private ventures, money-making in spirit and object" which should be "crushed with all the force that law and public opinion can bring to bear".

The report had the effect of a bombshell; in 1920, ten years after its publication, the number of medical schools in the United States and Canada had decreased to eighty-five; in 1930, to seventy-six. In the remaining schools a wholesome, continuing ferment of self-examination was created.

The text of Part 1 is a treatise on medical education; its status in the United States in 1910 and a programme for improvement. It was novel in 1910: it is far from obsolete now.

Space limitations permit mention only of the second Carnegie survey of medical education—that of Great Britain, France, Germany and Austria—made in 1910 and 1911 by Flexner and published in 1912 as Bulletin No. 6 of the Carnegie Foundation. Individual schools were not described in detail, only a picture of contemporary medical education in those countries being desired. The findings relating to Great Britain may perhaps be epitomized by quoting from an obituary notice in *The Lancet* of October 10, 1959: "It may not be too much to say that Flexner's ideas contributed to making medical education in this country something of which we may one day have reason to be proud".

Not only did the Flexner report accelerate the discontinuance of bad schools and stimulate the betterment of good ones, it also alerted philanthropic foundations other than the Carnegie to needs and opportunities in the field of medical education. In 1913 the Rockefeller-supported General Education Board, on advice from Flexner, allocated one and a half million dollars to the Johns Hopkins Medical School to be used to endow the chairs of medicine, surgery, pediatrics and obstetrics, so that their incumbents, relieved of the necessity of gainful practice, should devote their whole energies to teaching and research in the true university sense. This was the beginning of the 'full-time system' in clinical departments in the United States.

Also in 1913, Flexner was appointed to the administrative staff of the General Education Board; he later became secretary and director of studies. For fifteen years he guided the Board's allocations for medical education in such a way as to change its whole aspect in the United States. Those allocations amounted *in toto* to about 78 million dollars; they were distributed among medical schools of twenty-four universities, chosen because of ability and willingness to change. He developed remarkable skill in the technique of matching or contingent gifts, with the result that the 78 million dollars of Rockefeller money were made to add several hundred additional millions from other sources to American schools.

The results of these benefactions were by no means limited to the institutions to which they were made. The spirit of emulation caused many schools to do for themselves what otherwise would not have been done. In this sense the effects of Flexner's crusade became nation-wide.

In 1928 Flexner resigned from the secretaryship of the General Education Board, and its work in the field of medical education came to a close. During the spring of that year he gave the Rhodes Memorial Lectures at the University of Oxford, taking up residence in All Souls College during the term in which they were given. The entire following year was spent in gathering additional data and in rewriting the lectures previous to their publication as the book, "Universities: American, English, German". In addition to credit given for what was good, it contained outspoken criticism of "shameless humbuggery" in teachers' colleges and in home study and correspondence courses competing with on-the-campus work at certain named universities.

The book, as was doubtless expected, made a stir among educators; another result, unanticipated, was to determine the course of the next ten years of Flexner's life. This was the initiation of a succession of conversations with Mr. Louis Bamberger, a successful merchant of Newark, New Jersey, and his sister, Mrs. Felix Fuld, concerning philanthropies which they were in a position to support. This gave Flexner the opportunity to expound to them his long-held vision of what he later termed "a paradise for scholars, who, like poets and musicians, have won the right to do as they please and who accomplish most when enabled to do so". His exposition was so convincing that a grant of 5 million dollars was made to him with which to create what became the Institute for Advanced Study at Princeton, New Jersey.

The Institute was incorporated in May 1930 with Abraham Flexner, at the insistence of the founders, as the director. Mathematics was chosen as the first field of effort; Albert Einstein and Oswald Veblen were the first professorial appointments. It gives no scheduled courses; awards no degrees; admits only those judged to have "won the right to do as they please".

Now the Institute includes a School of Mathematics and a School of Historical Studies; there are fifteen permanent members who compose the faculty and fifteen other long-term members. There are at any one time about a hundred other members who are received for short periods—one or two years. They have come from all over the world. From the beginning of academic work in 1932 to the publication of a bibliography in 1955, the number of scholars who had taken advantage of the facilities was about 1,000; their publications of work done at the Institute were about 4,000.

Concerning himself in relation to the Institute, Flexner has written, "It seems odd to me that never until I met Mr. Bamberger and Mrs. Fuld did anyone dream that I could head an Institution. I was obviously regarded as too much given to innovation rather than administration. The charge was true, though misleading; for my feet were always on the ground, though always, it is true in motion".

"The director of the Institute must be constantly on the watch to prevent the invasion of the sort of routine and organization which may perhaps be indispensable to a university but would be death to the spirit of the Institute".

Flexner resigned the directorship in 1939, and was followed by Frank Aydelotte; he in turn, in 1947, by Robert Oppenheimer, the present incumbent.

In his autobiography, Flexner has made revealing comments on his qualities and on the circumstances which determined his career.

"I had in modest measure what Pasteur called the prepared mind".

"I cannot see that I have pursued anything but an opportunistic course".

"I was quick to absorb, eager to execute. I got what in most cases I wanted because I reduced what I wanted to what I believed I could get".

"I have cared—cared profoundly—about education, and especially higher education, since at nineteen years of age I began to teach. But my interest has always been pragmatic. I wanted to do something—to teach this or that or to enable someone else to do it".

He was exceedingly intelligent; confident in his convictions; ardently imaginative; open to the informed opinions of others; utterly fearless in his devotion to excellence; both fearless and happy in his struggles against mediocrity and sham. Although he disclaimed the possession of originality, the genius with which he utilized the variety of his opportunities produced unprecedented developments in American education.

A. N. Richards

NEWS and VIEWS

The J. Lawrence Smith Medal : Dr. E. J. Öpik

The J. Lawrence Smith Medal of the U.S. National Academy of Sciences, awarded for outstanding investigations of meteoric bodies, has this year been presented to Dr. Ernst J. Öpik, visiting research professor at the University of Maryland and director of the Armagh Observatory, Northern Ireland. Dr. Öpik is a pioneer in the study of meteors and their fiery trace through the Earth's atmosphere. On the basis of research performed in the early 1920's, he worked out a theory for the interaction of high-speed bodies with the upper atmosphere. His investigations were fundamental to an understanding of the ablation phenomenon—the peeling back of a meteor's surface as it vaporizes from the heat of atmospheric friction. Since the nose cone of a rocket plunging back into the Earth's atmosphere encounters the same destructive heat, Öpik's original principles and observations were of incalculable value to the designers of space vehicles in their search for solutions to the re-entry problem. Dr. Öpik's meteor studies have also contributed to a better understanding of comets and asteroids. In addition, he has made noteworthy contributions to other fields of astronomy and geophysics, among them studies of the planetary atmospheres of Mars, Venus and Jupiter. Considered by many to be his most significant is Dr. Öpik's theory of the interior structure of stars. Born on October 23, 1893, in Port Kunda, Estonia, Dr. Öpik studied astrophysics at the Imperial University of Moscow. He began his astronomical studies at Turkestan University in eastern Russia. He then served as astronomer and lecturer at the Tartu (Dorpat) Observatory in Estonia during 1921–44. From 1930 until 1934, he was research associate and lecturer at Harvard College and Observatory. Following the Second World War, he was professor of astronomy and Estonian rector at the Baltic University, Hamburg, Germany. In 1948, he was appointed research associate and astronomer of the Armagh Observatory in Northern Ireland, and has since become its director.

Sheep Husbandry and Wool Technology at Pretoria : Prof. D. M. Joubert

The appointment of Dr. D. M. Joubert to the chair of sheep husbandry and wool technology at the University of Pretoria has recently been announced. After studying for his M.Sc.(Agric.) at the University of Pretoria, Dr. Joubert worked for some three years in the School of Agriculture, University of Cambridge, under the direction of Sir John Hammond, and obtained his Ph.D. in 1956. On his return to South Africa he was appointed to a senior lectureship in animal husbandry in the University of Pretoria. Dr. Joubert's earlier work was on the influence of nutrition on the growth and reproductive performance of cattle. Since then, he has carried out detailed studies of some of the factors influencing both pre- and post-natal growth in sheep. He has used the analysis of the size of muscle fibres as an important tool in this work. With his broad interests in animal husbandry, meat quality and animal breeding, particularly in relation to sheep, Prof. Joubert should find that his new appointment offers ample scope for interesting developments in these fields.

Fisheries Research in Australia : Dr. G. Kesteven

Dr. Geoffrey Kesteven, chief of the Biology Branch, Fisheries Division, of the Food and Agriculture Organization, has been appointed director of fisheries research in the Division of Fisheries and Oceanography of the Commonwealth Scientific and Industrial Research Organization at Cronulla, New South Wales. Dr. Kesteven was responsible for the re-organization of the Biology Branch of F.A.O. to its present form. The branch's work has attracted international attention and is widely appreciated by existing fisheries commissions, councils and other inter-governmental bodies for the service it is rendering. One of his notable contributions was the organization of the intelligence service which is widely used in fisheries biological matters and the initiation of the "Current Bibliography for Aquatic Sciences and Fisheries". Dr. Kesteven first joined C.S.I.R.O. in 1939 as a junior research officer after two years with the New South Wales Fisheries Department. While still with that Organization, he was seconded to the office of the Commonwealth Controller of Fisheries in 1942, work with which he maintained association until 1947, when he joined the Food and Agriculture Organization as regional fisheries officer for the Far East. In 1952 he went to Rome as a member of the Biological Branch ; he was appointed branch chief in 1954. Mr. J. S. Holt succeeds him as biology branch chief.

Colonial Research Committees

In a written answer in the House of Commons on March 29, the Minister of State for Commonwealth Relations, Mr. C. J. M. Alport, said that apart from the Colonial Research Council and the Colonial Products Council, all the research committees which had advised the Colonial Secretary on the expenditure of Colonial Development and Welfare Funds allocated to research would continue to do so. The Overseas Research Council had now taken over from the Colonial Research Council the function of advising on general research policy and co-ordinating the work of the advisory committees. There are at present no standing sub-committees of the Council itself.

Feeding Antibiotics to Farm Animals

The Agricultural and Medical Research Councils have set up a Joint Committee to examine the possible consequences of feeding antibiotics to farm animals and to consider whether this use constitutes any danger to human or animal health. The Committee will make such recommendations to the two Councils as may be thought necessary and its report will be made available through the Councils to the Ministers concerned. Lord Netherthorpe, a member of the Agricultural Research Council, has accepted an invitation to act as chairman, and Prof. A. A. Miles, the director of the Lister Institute of Preventive Medicine, has agreed to take the chair of a Sub-Committee which will examine the evidence in detail and place its findings before the main Committee. Apart from medical and veterinary scientists, the membership of the Committee includes farmers and experts in animal nutrition.

The Caribbean Commission

THE Caribbean Commission is a consultative and advisory body established in October, 1946, by the Governments of the French Republic, the Kingdom of the Netherlands, the United Kingdom and the United States. The Commission has as its main object the economic and social well-being of the territories of the Caribbean associated with the member governments, and concerns itself with matters of common interest to the area, particularly agriculture, communications, education, fisheries, health, housing, industry, labour, social welfare and trade. There are two auxiliary bodies of the Commission: the Caribbean Research Council and the West Indian Conference. The Research Council considers the research problems of the area and recommends action to the Commission, including the undertaking of research assignments. The West Indian Conference provides regular means of consultation with and between delegates from the territories on matters of common interest. The Conference is convened by the Commission at least biennially. The Commission maintains a Central Secretariat at Port-of-Spain, Trinidad, and publishes a monthly journal, *The Caribbean*, which is printed in English and French. The main article in a recent issue (**13**, No. 7: July 1959) dealt with problems of over-population, particularly with the way in which economic development is being promoted to resist demographic pressure.

Training of Technical Teachers

THE Northern Advisory Council on Further Education has continued its examination of the problems of establishing a group of tutors, the function of which would be to organize, or assist in organizing, courses of training for technical teachers in the region, such courses being normally of limited duration. The Council has agreed that such a system should be established, but the most appropriate means of bringing it into operation has yet to be determined. With a grant from the Gulbenkian Foundation, Mr. A. Pennington, lecturer in the Department of Education of the Durham Colleges, was released for the session 1958–59 to work under the general guidance of the Council's Programme Committee, and he has visited colleges for observation of teachers in classwork. In addition, Mr. Pennington has submitted valuable memoranda to the Programme Committee on the future arrangements for training technical teachers in the region, based upon inquiries in the region and other parts of Britain. Details of these courses and information about the work of the Council are to be found in the report for 1958–59. The Secretary's address is 5 Grosvenor Villas, Newcastle upon Tyne 2.

Teaching Within the British Commonwealth

IN a "Teachers for the Commonwealth" Conference at Westminster on February 23, at which Sir David Eccles referred to the four hundred additional teachers which the United Kingdom proposed to make available for key posts in the Commonwealth, Mrs. V. L. Pandit, the High Commissioner for India, stated that when measures for introducing compulsory and universal education for children in the age group 6–11 were initiated by the beginning of the third five-year plan, 20 million more children would be involved and 500,000 trained teachers required. At the primary level, recruitment would have to be from within India, but at higher levels teachers from Great Britain or the Commonwealth would be of real service, and particularly in teacher training and in subjects such as English, social studies and technical training. The Commissioner for Nigeria, A. A. Maliki, said that in the Federation of Nigeria there was a shortage of four hundred graduate teachers, half the vacancies being in science, and a fair number in English. It would be a long time before graduates in its schools could become more than a nucleus around which nongraduate teachers would cluster, and accordingly their energies must in the first instance be directed to training large numbers of nongraduate teachers. Developments in technical education in Nigeria were mostly pre-war and it had been impossible to keep pace with the demand for technologists, technicians and craftsmen. The staffs at trade centres and technical institutes were almost entirely expatriates and were becoming more and more difficult to recruit. With a population of more than 35 million, Nigeria had 1,200 students in a university college, 1,200 pursuing professional training in a college of arts, science and technology, 20,000 in teachers' training colleges, 40,000 pupils in secondary schools, and 2·5 million in primary schools.

The National Parks Commission

A NUMBER of important topics discussed in the tenth annual report of the National Parks Commission, covering the year ended September 30, 1959, have already been discussed in *Nature* (see **184**, 1005; **183**, 917; 1959), and for the rest the report records a year of activity on the usual lines (pp. vi+63+8 plates. London: H.M. Stationery Office, 1959. 6*s*. net). Against continued concern with the existence of firing ranges in National Parks, the encroachment of development proposals including water schemes, particularly in Devon, nuclear power stations, aerial masts, and afforestation, and the unsatisfactory administrative arrangements in, for example, the Yorkshire Dales National Park, are to be set the success of the Peak District Board in tree planting and improving woodlands, its voluntary warden service, and with removal of derelict buildings in the Lake District and the great efforts being made in Snowdonia to mitigate damage done to the landscape by large-scale industrial development. One of the most important questions with which the Commission was concerned during the year related to the Buckland Court Estate Woods in the Dartmoor National Park which were the subject of a Tree Preservation Order made by the Devon County Council to which the owners objected. The Commission was consulted by the Minister of Housing and Local Government, but its recommendations were not accepted, and in the Commission's judgment an opportunity for creative landscape afforestation has been missed. Increasing attention is being given to publicity, but the Commission still needs increased appropriations and staff resources for its information work, and in conclusion the Commission again directs attention to the over-riding problem of adequate finance if the potentialities of the National Parks are to be realized, and our national heritage in the countryside preserved and enhanced in the face of continuing threats from other interests. Over the past ten years less than one penny per person per year has been spent in connexion with national parks in Britain.

Animal Health

THE Animal Health Yearbook, 1959 (pp. 201+vi. Rome: Food and Agriculture Organization of the

United Nations, 1959. 10*s*.), is the third of the annual reports on the diseases of livestock of the world that have been issued. It contains information available up to the end of 1958, and is compiled from replies to questionnaires sent by FAO/OIE to the veterinary services of their respective countries. The countries are listed in geographical order in the table of contents, and the diseases mentioned are arranged in seventeen tables, their names being given at the top of each page in English, French and Spanish, together with the Latin name for each disease, and in many instances the causal agent as well. Succeeding the tables, which occupy the bulk (175 pages) of the report, is a valuable map which shows the distribution in the Americas of foot-and-mouth disease and vesicular stomatitis, and articles follow on the global position and control of important contagious diseases of animals and on the Pan-American Zoonoses and Foot-and-Mouth Disease Centres. The diseases discussed include foot-and-mouth disease, rinderpest, Newcastle disease, hæmorrhagic septicæmia, blue-tongue, mastitis and tuberculosis. A valuable article by J. N. Ritchie, chief veterinary officer to the British Ministry of Agriculture, Food and Fisheries, discusses the remarkable progress made in the eradication of tuberculosis in Britain and America and the methods by which this progress has been made. All these articles are printed in English, French and Spanish. The report concludes with an exhaustive index, which enables the reader to find the detailed information he may require. Printed in small type by offset lithography on pages that measure about 11½ in. × 9½ in., the report is certainly somewhat large to handle; but the large page and the clarity of the type undoubtedly make it easy to read.

Journal of the Institute of Biology

After six years of steady growth and development from a tentative and modest beginning, the *Journal of the Institute of Biology* has been given a new cover, format, type and paper. To ensure that the improvement is not merely superficial the number of editors has been increased from two to three. Mr. R. W. Marsh is the new editor, and with his wide experience of biological publications and the more applied aspects of biology, he should greatly strengthen the *Journal* and broaden its scope. There is no intention of changing the general policy of the *Journal*. The object is not to provide another outlet for research papers, but to supply a medium for the exchange of information and ideas about the profession of biology and for the discussion of the more general aspects of the subject which are outside the scope of the specialist periodicals.

In a recent issue (**7**, No. 1; January 1960) it is reported that, in the summer of 1959, 100 candidates sat examinations for the Endorsed Certificate at the Ordinary Level. The examinations were held in nine colleges and 65 people were successful. The Higher Endorsed Certificate was taken in five centres by 31 people of whom 27 were awarded certificates. Another interesting item concerns the supply of biologists. The estimated number of biologists required in 1962 is 8,000. This means that there are likely to be 1,200 new posts in the next three years; in addition there will be a need for at least 510 biologists to replace those of the present 6,800 who reach retiring age. The minimum number of biologists required in the next three years would seem to be 1,710. At present 490 people take honours degrees in biology each year. The production of biologists in the next three years is likely to be 1,470; of these nearly 600 will be women, and marriage is likely to take a proportion of these away from the profession.

The Biophysical Journal

The Biophysical Society and the Rockefeller Institute Press are to publish a new journal—*The Biophysical Journal*. The first volume will include eight bimonthly issues beginning with September 1960, and subscriptions will be eight dollars to non-members. Members who subscribe through the Biophysical Society will receive a discount. After the first volume the *Journal* will appear in one volume a year of six issues each, beginning in January, at a subscription price of six dollars to non-members. Manuscripts and subscriptions are now being accepted. Manuscripts should be addressed to the editor, Prof. Frank Brink, jun., Rockefeller Institute, New York, 21.

School Natural History Societies

With much in common, reports from the natural history societies at Oundle, Dauntsey's and Eton College reflect what can be done by schoolboys taught by enthusiastic staffs. At Oundle, J. V. Oubridge investigated the reasons for the decline in the number of frog embryos from the time of laying until they become adults in the (unfortunately named) Cemetery Pond. This involved confirming that a decrease does, in fact, take place, estimating it quantitatively, finding out what kills the embryos and whether or not the mortality is selective or random. D. K. Hastings, of Dauntsey's School, describes an investigation made during his attendance at a specially arranged ecology course at Anglesey during the Easter vacation, 1959. He examined the ways in which the morphology and density of *Pelvetia canaliculata* and *Fucus spiralis* might differ in sample areas chosen for their degree of shelter and exposure, respectively. At Eton, in 1958, the Natural History Society Prize was awarded to P. N. Buckley, who submitted an account and specimens of an extensive collection of moths he had made with a mercury vapour lamp trap. His work continued in 1959, and it is hoped a full report will be published in 1960, with a list of species, including melanic and other varieties.

Directory of Radioisotopes

Relatively few countries have as yet been able to take full advantage of the social and economic benefits derived from the use of radioisotopes. Accordingly, the International Atomic Energy Agency aims to assist member countries to acquire the knowledge and skills needed to make full use of these important materials. As part of its programme, the Agency has recently published a Directory of Radioisotopes, giving information about sources of supply, methods of procurement and prices (International Directory of Radioisotopes. Vol. 1: Unprocessed and Processed Radioisotope Preparations and Special Radiation Sources. Pp. ix+264. 73.50 schillings, 21*s*., 3.50 dollars. Vol. 2: Compounds of Carbon-14, Hydrogen-3, Iodine-131, Phosphorus-32 and Sulphur-35. Pp. vi+211. 63 schillings, 18*s*., 3 dollars. London: H.M. Stationery Office; and Vienna: International Atomic Energy Agency, 1959). The first part contains tables of all the radioisotopes which are offered

for sale by any of the international suppliers, including processed and unprocessed preparations, and solid radiation sources for particular purposes. The second part deals with chemical compounds labelled with carbon-14, tritium, iodine-131, phosphorus-32 and sulphur-35. The isotopes and their compounds are listed alphabetically, and brief technical and commercial data are given for each item.

It is claimed that these volumes are comprehensive. They certainly give extensive coverage of this subject: there are listed some 185 different isotopes, a very large number of labelled compounds, including more than one thousand for carbon-14 alone, and reference is made to forty-four suppliers, being either national or commercial enterprises and operating in about a dozen different countries. For current prices (those in the Directory are no later than 1958) and fuller technical information it will still be necessary for users to consult the suppliers directly; but as a directory this publication will undoubtedly be most useful, and it makes plain the very great resources of radioisotopes and radioactive sources which are now readily available to all who are able to profit by their use.

Treatment of Injured and Regenerating Muscles with Plant Extracts

In recent years, many research workers in Czechoslovakia and elsewhere have examined the use of extracts from higher plants and have studied their effect not only on micro-organisms, but also on macro-organisms (*Folio Biologica* (Praha), 5, No. 6; 1959). Soviet authors in particular have studied the possibilities of influencing healing processes with these extracts, since they contain phytoncides, substances which, in addition to bacteriostatic and bactericidal properties, are also capable of stimulating healing processes in injured tissues or organisms. Certain phytoncidal substances found, for example, in onion or garlic extracts, stimulate regeneration processes and markedly accelerate the healing of wounds. The successful results obtained by some investigators with the use of plant extracts in wound-healing stimulated S. Hoja and J. Vittek to make an experimental study of the effect of these substances on the course of regeneration processes in injured and transplanted muscles. Parsnip, fir, garlic and onion extracts were injected over long periods into injured and transplanted striated muscle in rabbits, and their effect on morphological processes in the muscle was studied. Not one of the test substances was found to stimulate regeneration of the muscles.

New Record Low Sea-level Air Pressure

A pressure at sea-level of 877 mb. (25·91 in mercury) was recorded by a radiosonde dropped from a U.S. Air Force Weather reconnaissance aircraft into the eye of a typhoon in the western Pacific near Guam on September 24, 1958. The previous lowest value was 886·7 mb. (26·185 in.) observed on a ship in a typhoon in 1927. Full details of the observation including reasons for believing the value to be accurate to within 4 mb. are given in an article by C. L. Jordan in the *Monthly Weather Review* for September 1959. Sea-level pressures below 900 mb. have been observed in seven other typhoons in the past eight years. The normal value of pressure at sea level is 1,013 mb. and a pressure of 877 mb. occurs normally at a height of about 3,700 ft.

Gordon Research Conferences, 1960

Every Sunday, from June 12 until August 28 inclusive, a similar scene will be enacted in three residential schools in New Hampshire, when about a hundred scientists will be arriving to register their attendance at a Gordon Research Conference. From Monday morning until Friday midday they will take part in morning and evening sessions in which a very few lectures and much discussion will take place under very friendly, relaxed and informal conditions. The discussions will be wide-ranging and profound, many new ideas and facts will be freely exchanged, because each participant knows that any information which he divulges will not be used without his specific authorization and that no written records will be kept. These conferences are among the most successful and enjoyable which have yet been organized. They are held in great esteem, requests for attendance always greatly exceed the number of places and different groups compete keenly to have their subject as one of those selected for one of the conferences. European scientists are now showing signs of paying the Gordon Conference organizers the compliment of trying to organize conferences along similar lines in Europe. Radiation chemists organized the Miller Conference in 1959 and polymer chemists hope to hold their first conference in 1961.

The topics of the thirty-six Gordon Research conferences to be held this summer are still mainly chemical, and are either of long-term importance (such as corrosion, catalysis, inorganic chemistry) or represent growing points in the subject (such as high-pressure research, high-temperature chemistry). Full details of dates, topics, costs, registration and reservations for this year's conferences can be obtained from Dr. W. George Parks, Department of Chemistry, University of Rhode Island, Kingston, R.I., U.S.A.

Lederle Medical Student Fellowships

The Lederle Laboratories Division of the American Cyanamid Co. announces that it is making available fellowships to medical schools throughout the United States and Canada for the year 1960. These fellowships, in amounts not exceeding 600 dollars a year for any one individual, are intended to relieve in part the financial burden of students who desire to devote their summer vacations to research in the preclinical departments. Students who apply for Lederle Medical Student Research Fellowships must have the consent of the faculty member under whose supervision their research is to be conducted. Further information can be obtained from the Lederle Laboratories, American Cyanamid Co., Pearl River, New York.

Carlsberg-Wellcome Travelling Research Fellowships

Applications are invited for the Carlsberg-Wellcome Travelling Research Fellowships, 1960–61, the object of which is to encourage co-operation, on an exchange basis, between Danish and British research workers in any branch of the natural sciences which has a bearing upon human and animal medicine. One Fellowship annually is awarded to a candidate from the United Kingdom for a year's work in Denmark and one annually to a Danish candidate for a year's work in the United Kingdom. The stipend may range from £900 to £1,300 per annum (or the equivalent sums in Danish kroner), travelling and some incidental expenses being provided in addition.

The tenure of the awards will start in September 1960. Further information can be obtained from the Assistant Scientific Secretary of the Wellcome Trust, 52 Queen Anne Street, London, W.1. Applications must be submitted before April 30.

The Institution of Agricultural Engineers: Bursaries and a Scholarship

The Institution of Agricultural Engineers is offering, through the generosity of the Dunlop Rubber Co., Ltd., a postgraduate scholarship for entry to the final year's course of study leading to the examination in July 1961 for the National Diploma in Agricultural Engineering. Shell Mex and B.P., Ltd., have enabled the Institution to offer a number of bursaries. The value of the Dunlop Scholarship is £250, and the bursaries are normally available in units of £50. Further information can be obtained from the Secretary, Institution of Agricultural Engineers, 6 Queen Square, London, W.C.1.

University News: Bristol

Dr. A. S. King (veterinary anatomy) and Mr. H. Sutcliffe (electrical engineering) have been appointed to senior lectureships. Mr. J. S. Cooper has been appointed as lecturer in prosthetics in the Department of Dental Surgery. Grants have been received as follows: £25,000 from the Research Grants Advisory Panel of Nato to the Department of Physics for the support of an international expedition to the central Mediterranean; £6,500 from the Department of Scientific and Industrial Research for the three years ending April 30, 1963, to the Department of Physics in connexion with an investigation of high-altitude flight and recovery techniques in the Mediterranean region and £1,600 to the Department of Organic Chemistry for an investigation into the structure and synthesis of natural products; £2,200 from the Department of Scientific and Industrial Research for the year ending December 31, 1960, towards research in the Department of Physics on the study of fragmentation of heavy cosmic ray nuclei in hydrogen and carbon; £2,000 from the Nuffield Foundation for work in the Department of Surgery on vascular disease for one year; £1,250 per annum for two years from Roche Products, Ltd., to the Department of Organic Chemistry for the establishment of a Roche fellowship for research work.

Southampton

A contribution of £20,000 to the 1962 Centenary Appeal Fund has been received from Shell-Mex and B.P. Ltd.

Mr. A. M. MacIver, head of the Department of Philosophy, has been appointed to the newly established chair of philosophy. The title of senior lecturer has been conferred upon Dr. H. G. Jerrard (physics), Dr. G. A. Kerkut (physiology and biochemistry), Dr. G. R. Lane (botany), Mr. S. W. Punnett (electronics). The following new appointments to lectureships have also been made: Dr. R. J. Gribben and Dr. L. Marder (mathematics), Mr. H. S. Stachera (electronics), Dr. I. D. R. Stevens (chemistry) and Mr. T. C. Whitmore (botany).

The Night Sky in May

Full moon occurs on May 11d. 05h. 43m. U.T., and new moon on May 25d. 12h. 27m. The following conjunctions with the Moon take place: May 14d. 01h., Jupiter 5° S.; May 15d. 03h., Saturn 4° S.; May 20d. 22h., Mars 0·1° S. In addition to these conjunctions with the Moon, Mercury is in conjunction with Venus on May 6d. 02h., Mercury being 0·2° S. Mercury, Venus and Mars are too close to the Sun for easy observation. Jupiter rises at 23h. 40m., 22h. 40m. and 21h. 35m. on May 1, 15 and 31, respectively. It is in Sagittarius, its stellar magnitude is −2·1 and its distance from the Earth in the middle of the month is 410 million miles. Saturn is also in Sagittarius, rising at 0h. 40m., 23h. 40m. and 22h. 40m. on May 1, 15 and 31, respectively. Its stellar magnitude is +0·6 and its distance from the Earth on May 15 is 880 million miles. There are no occultations of stars brighter than magnitude 6 visible at Greenwich.

Announcements

H.M. Queen Elizabeth the Queen Mother will open the Brunswick Square building of the School of Pharmacy, University of London, on April 27, at 2.30 p.m.

Dr. M. R. J. Salton, reader in bacteriology in the University of Manchester, will give the annual Ciba Lectures in Microbial Biochemistry at the Institute of Microbiology, Rutgers, The State University, during April 25–27. His subject will be "Microbial Cell Walls".

The British Rayon Research Association is organizing open days during May 11–13 at the Heald Green Laboratories, Wythenshawe, Manchester. Further information can be obtained from the Information Officer at the Laboratories.

A general symposium on Arid Zone Problems is being organized by Unesco and will be held in Paris during May 11–18. Further information can be obtained from M. Batisse, Arid Zone Unit, Natural Sciences Department, Unesco, Place de Fontenoy, Paris 7e.

A joint conference on Physical and Structural Aspects of Non-destructive Testing of Metals will be held by the Non-destructive Testing Group of the Institute of Physics and the Société Française de Métallurgie during May 2–4. The conference will be held in the Lecture Theatre of the Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1. Further information can be obtained from Mr. L. Lawrence, Institute of Physics, 47 Belgrave Square, London, S.W.1.

A major scientific conference to be arranged by the International Atomic Energy Agency in 1960 will be held in Copenhagen during September 6–17. The conference will deal with the use of radioisotopes in the physical sciences and industry, and it is expected that more than five hundred scientists from the Agency's seventy Member States will attend.

La Société de Chimie Physique announces that the meeting on the Structure of Graphite and the Kinetics of its Reactions (see *Nature*, 185, 582. February 27) has been postponed for a week, and will now be held during June 7–10. Further information can be obtained from the Secrétaire Général de la Société de Chimie Physique, 10 Rue Vauquelin, Paris-Ve.

It is stated in a review of Bayliss's "Principles of General Physiology" in *Nature* of April 2, p. 52, that the price of Vol. 2 is 90*s*. net; Messrs. Longmans, Green and Co., Ltd., inform us that the price will be 85*s*. net.

INDUSTRIAL CHEMISTRY OF THE LOWER OLEFINES

EVERY year Manchester is fortunate in having a symposium under the joint auspices of the Chemical Society, the Institute of Petroleum, the Royal Institute of Chemistry, and the Society of Chemical Industry. The symposium, held on March 24, entitled "The Industrial Chemistry of the Lower Olefines", and organized by the Institute of Petroleum, fully maintained the high standards set in earlier years.

It is good to take stock periodically, and this symposium was essentially an appraisal of the current position relating to the compounds ethylene, propylene, butadiene and ethylene oxide, and to the methods more recently available for the conversion of simple olefines to polymers of technical importance. There were no startling new revelations, but such are not expected in a stocktaking process.

The symposium was divided into a morning and afternoon session, each of two lectures, and an early evening session of one lecture. The morning session, under the chairmanship of Prof. F. Morton, began with a detailed and useful paper by J. Chrones and J. L. James (Kellogg International Corporation) entitled "The Economics of Ethylene Production from Light Naphtha". Without the knowledge that the preprint of his paper had been issued in advance, it would have been quite impossible for Mr. Chrones to have presented verbally the wealth of detail and calculation in his paper within the time available. It was predicted that in regions such as Britain, where natural gas is not available, light naphtha will continue to be the favoured raw material for the production of ethylene by steam cracking. Light naphtha is available readily and at a relatively low price; this price and the value of the cracking products other than ethylene (for example, propylene, butadiene, gasoline, fuel oil) form the main factors affecting the economics of ethylene manufacture. These economics were considered for three types of light naphtha pyrolysis units designed to yield 100 millions of pounds per annum of polymer-grade ethylene of 99·9 per cent purity: (*a*) high-severity pyrolysis with recycle of ethane product to pyrolysis, thus minimizing feed consumption, and favoured when the cost of light naphtha is high, (*b*) medium severity pyrolysis with recycle of ethane to pyrolysis, and (*c*) medium severity pyrolysis without recycle of ethane, this being preferred when the light naphtha cost is low or the value of the by-products is high. Under present conditions the demand for ethylene as a petrochemical considerably outweighs that for the other by-products; pyrolysis under conditions favouring a high ratio of propylene to ethylene thus cannot be justified, since enough propylene is obtained as by-product under high-severity pyrolysis conditions to satisfy completely the increasing demand for propylene for conversion to polypropylene.

Numerous articles have appeared on the manufacture of ethylene oxide, and it is a credit to Dr. R. P. van Oosten (Bataafse Internationale Chemi Maatschappij) that his approach to the subject "The Production of Ethylene Oxide", and the style and delivery of his lecture, were refreshingly original. Two processes are of technical importance at the present time: the chlorohydrin method, and the one-step direct oxidation using oxygen in presence of a silver catalyst. In 1955 some 55 per cent of the ethylene oxide in the United States was prepared by the chlorohydrin route; in 1960 only 40 per cent will be so prepared. New producers of ethylene oxide thus prefer the direct oxidation route, and the older chlorohydrin plants are often being used for the production of propylene oxide, a chemical of increasing importance, and one for which no satisfactory direct oxidation process from propylene at present exists. The chlorohydrin method for ethylene oxide production gives a better overall yield, but suffers from two major disadvantages: chlorine is required, and increase in chlorine production aggravates the growing unbalance between chlorine and caustic soda production; furthermore, by-product ethylene dichloride and waste-product calcium chloride have to be disposed of. Most of the engineering difficulties at one time apparent in the direct oxidation process have now been simplified or solved, and the facts that concentrated ethylene, necessary for direct oxidation, is becoming a relatively cheap chemical, and that the yields of ethylene oxide and the life of the silver catalyst steadily increase, emphasize still more the reasons for the emergence of the direct oxidation route as the preferred method. With a total consumption in the United States of 910 millions of pounds per annum in 1955 rising to an estimated total of 1,250 millions of pounds per annum in 1960, and 1,800 millions of pounds per annum in 1965, few chemicals have a brighter future than ethylene oxide.

The afternoon session, under the chairmanship of Prof. R. N. Haszeldine, got off to a good start with the paper by Dr. A. F. Millidge (Distillers Co., Ltd.) on "Industrial Chemicals based on Ethylene and Propylene". This is an enormous topic and the paper was correspondingly by far the longest of the symposium. The main lessons to be learned from this detailed review were, first, the versatility of ethylene and propylene in providing industrial raw materials and, second, that even to-day it is still possible to discover quite new reactions and technical processes. Formation of acrylonitrile by reaction of propylene with ammonia and air; direct oxidation of ethylene to acetaldehyde; preparation of ethyl acetate in 98 per cent yield directly from acetaldehyde; conversion of propylene into acrylonitrile by use of nitric oxide; oxidation of *iso*propyl alcohol to acetone and hydrogen peroxide, provide typical examples.

The next paper, by Mr. A. A. Appleton (Esso Petroleum Co., Ltd.), was entitled "Production and Utilization of Butadiene". It reviewed first the historical development of butadiene as a petrochemical, then dealt with methods of preparation. Particular attention was paid to routes involving catalytic dehydrogenation of the butenes. Steam, used as diluent, provides a medium which both carries heat to the catalyst bed and simultaneously lowers the butene partial pressure—very necessary if polymerization and ultimate degradation of butadiene to coke and hydrogen are to be avoided. Several types of catalyst were compared, the most selective being a

calcium nickel phosphate stabilized with chromium oxide. Conversions of butenes to butadiene of 30–50 per cent can be obtained with a selectivity of approximately 90 per cent. Other catalysts, for example, one containing ferric oxide, chromium oxide and potassium oxide, are less selective at higher conversions, but require a lower steam dilution ratio and have longer life.

The direct dehydrogenation of *n*-butane to butadiene is being used at five of the more recent butadiene plants. A particular advantage of this process, employing a catalyst of activated alumina with 18–20 per cent of chromic oxide, is that it can be used to make butadiene or a mixture of butadiene and butenes. The present position in Europe is such that supplies of butenes from refinery catalytic cracking are inadequate, and emphasis has been placed, for example, in Germany, on butane dehydrogenation; the European figures for butadiene (18 per cent from butenes, 22 per cent from *n*-butane, 38 per cent from steam cracking of light naphtha and 22 per cent from acetylene), are quite different from the corresponding figures from the United States (48, 41, 11 and 0 per cent, respectively). The heavy European dependence on steam cracking is associated with the considerable ethylene production capacity based on this route, and this position is unlikely to change for some time, although additional butadiene plants will need to be built to cope with the demand for SBR rubber.

Despite its versatility as a chemical, butadiene is too expensive an olefine compared with ethylene or propylene to find major outlet in anything but the polymer field; even acetylene is slightly less expensive. In the United States, 85 per cent of the butadiene is used in the manufacture of SBR rubber, with much smaller amounts (each 2 per cent) in the oil-resistant butadiene–acrylonitrile synthetic rubber, and in production of adiponitrile for nylon. Greatest growth-rate is offered by the polybutadiene rubber process, one involving stereospecific polymerization by Ziegler catalysts to a predominantly *cis* 1,4-structure; this elastomer approaches natural rubber in properties although, unlike *cis* 1,4-poly*iso*prene, it must be used admixed with natural rubber.

The final symposium paper, "Newer Developments in the Polymerization of Olefines", was delivered by Prof. C. E. H. Bawn, with Prof. G. Gee in the chair. Prof. Bawn showed how a difficult, controversial and rapidly developing field of study, initiated by the now-classical work of Ziegler and Natta, could be summarized and presented in a clear and logical manner. The scientific significance and the commercial importance of the catalytic methods for the production of new classes of sterically regular polymers are apparent to everyone; seldom has one discovery had so rapid and enormous an impact on an industry. After surveying the Ziegler-type catalysts and the catalysts of the supported metal oxide type for the low-pressure polymerization of ethylene and other olefines, Prof. Bawn went on to discuss in masterly style the structures of the olefine and diene polymers and their properties. This paper brought to a close a most successful symposium.

Interest in all the papers was high, and the audience had an appreciable number of questions to ask. Drs. H. Kaye, P. J. King and C. A. Curtis are to be congratulated on their organization; even Manchester provided blue skies for the occasion. The papers and discussion, which will appear in the *Journal of the Institute of Petroleum*, provide useful reading for all chemists and chemical engineers, since interest in the lower olefines extends far beyond the petroleum or petrochemicals industry. These compounds are the key building blocks of modern industrial organic chemistry.

R. N. Haszeldine

THE NATIONAL INSTITUTE OF GENETICS, JAPAN

In 1949 the Institute of Genetics was set up in Japan to cover practically the whole range of this discipline. There are six departments devoted to: morphological genetics, cytogenetics, physiological genetics, biochemical genetics, applied genetics, and induced mutation. In addition to these, the Rockefeller Foundation supports two groups of workers, one in special rice studies, and the other on the effects of radiation in animals.

Fifty-one research projects are being pursued in the six departments*. These range from virus, through insects (silkworms and drosophila) and plants to mammalia. They include such diverse topics as the cytology and genetics of tumours to studies of right- and left-handedness in plants. Radiation studies play a large part throughout the programme.

Many contributions deserve extended notice, though space will permit casual reference to only one or two of them.

An interesting paper is on the subject of a silkworm poison emanating from tobacco plants. It has long been known that when silkworm larvæ are fed on leaves taken from mulberry trees near a field of tobacco they are often poisoned. Tsujita and his co-workers now show that the poisonous substance is nicotine, which is present in mulberry leaves to the amount of 1–10 mgm. per kgm. When mulberry leaves were painted with diluted nicotine solutions of this order of concentration, silkworm larvæ showed similar symptoms of poisoning to those observed in the field. It is believed that nicotine molecules emanate from the tobacco plant secretions or from the disintegrating cells of the leaf hairs.

Cereal workers will note with interest the work of Oka and Sakai on the mode of inheritance of competitive ability in rice. They show that the competitive ability of a strain is determined genetically, and can be analysed by the same method as used for ordinary quantitative characters.

Differences in radiosensitivity and radiomutability between cultivated and wild strains of the Japanese morning glory were investigated by Kihara and Sakamoto. They report that both radiosensitivity and radiomutability of the cultivated strains was higher than in the wild one.

The number of papers published by the staff of the Institute was 108 in 1958, and 65 in 1957.

S. C. Harland

* National Institute of Genetics, Japan. Annual Report, No. 9, 1958. Pp. v+144. (Misima, Sizuoka-ken: National Institute of Genetics, 1959.)

THE NATIONAL INSTITUTE OF AGRICULTURAL BOTANY

ONE of the objects of the National Institute of Agricultural Botany is promoting the improvement of existing varieties of seeds, plants and crops in the United Kingdom and aiding the introduction or distribution of new varieties, and the annual report recently published indicates the wide range of work being undertaken to further this object*.

The work of the Trials Branch resulted in the addition of six varieties of cereals to the recommended list, while fifty-eight varieties were not approved. Short notes are given on the performance of many of these. Similar reports are given on preliminary results for other crops, and in the vegetable trials special attention has been paid to varieties suitable for quick freezing. Yield trials on recently introduced and foreign varieties of potato were continued at various centres. Progress is reported in attempts to explain stem-end blackening in the cooked potato. These chemical investigations, being conducted in collaboration with the Low Temperature Station, Cambridge, have shown that differences in *p*H have much less effect on the amount of discoloration than differences in the concentration of citric acid. It has also been demonstrated that the increase in discoloration in stored potato is associated with a sudden decrease in citric acid when sprouting begins.

The Seed Production Branch records considerable progress in the development of the arrangements for seed approval and certification. Almost 130,000 acres were inspected under the Cereal Field Approval Scheme. The report of the Official Seed Testing Station directs attention to some interesting differences in the proportions of varieties of cereals which failed to reach the minimum authorized percentage germination for declaration as laid down by the Seed Act. The varieties Cappelle Desprez and Koga II both represented about 25 per cent of the wheat samples reaching the Seed Testing Station, while Proctor represented nearly 50 per cent of the barley samples. One of the investigations in progress in the Seed Testing Station is concerned with the persistence of weed seeds in the soil and factors concerned in their germination and establishment. Detailed reports have been prepared of the investigations into the role of oxygen in the dormancy of wheat varieties and of the effects of maturation on the embryo, the endosperm and the covering layers. A comparative study is in progress of the maturation in two barley varieties, Domen and Herta, which differ markedly in their germination behaviour at harvest.

* National Institute of Agricultural Botany. Thirty-ninth Report and Accounts, 1958. Pp. 56. (Cambridge: National Institute of Agricultural Botany, 1959.)

AUTOMATIC CONTROL IN SOVIET INDUSTRY

THE Department of Scientific and Industrial Research has issued a report giving the observations of a team of six British engineers who visited the Soviet Union in May 1959 to study progress in automatic control of industrial production (Automatic Control in Soviet Industry. Pp. 64. London: Department of Scientific and Industrial Research, 1959).

Besides industrial establishments, the team visited research institutes under the Academy of Sciences, U.S.S.R., the Central Laboratory for Automation, Moscow, various educational establishments, the Computing Centre of the Mechanized Faculty, University of Moscow, and the Computer Centre of the Academy of Sciences, Moscow. The team considered its chief function to be the opening of channels of communication and the establishment of personal relations, and in this the visit appeared to be most successful. A few firm conclusions were drawn from what was seen. Work is in progress in most sections of the field of industrial control systems and most distinctive classes of device are in process of development. Analogue computers are freely used and digital machines are rapidly becoming easily available, though generally this work appears to be less advanced than in Britain. Considerable activity was noted on control in the steel industry, particularly in developing integrated process-control simulators, and work is also well advanced in telemetering and on the control of electric power systems with long transmission lines. Less attention appears to be paid to project evaluation than is usual in industrial research in Britain.

Great potential is being built up for further developments in automatic control and automation in industry in the form of large well-equipped laboratories and trained engineers. Of five research institutes visited, two had been established for only two years and one for three, and the Central Institute for Automation of the Ukraine at Kiev plans to increase its existing staff of 700 to 4,000 in three to four years. Some 83,000 students were said to have graduated in 1958, and the connexion between teaching, research and industry is good, with much interflow of staff. The team gained the impression that in a few years the application of automatic control in Soviet industry will have overtaken that in Britain, but there is at present little evidence to judge how good Soviet engineers will prove in the further stage when leadership depends upon breaking new ground, though their present level of achievement in basic theoretical work is high.

The report stresses the desirability of extending communications with the U.S.S.R. in this field, the importance of more British engineers acquiring sufficient knowledge of Russian to use Soviet technical literature, and the friendliness with which the team was received. Some suggestions are made regarding the arrangement of future visits.

GEOMETRY OF THE SOUTHERN AURORAL ZONE AND THE EVIDENCE FOR THE EXISTENCE OF AN INNER ZONE

By Dr. T. HATHERTON

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In 1955 Alfvén[1] developed his electric field theory of magnetic storms and auroræ taking into account the inertia of the particle beam which in a previous discussion[2] he had neglected. A consequence of this new work was the prediction of an inner auroral zone at a polar distance of 5–10°. Recently Lassen[3] has directed attention to a population of auroræ which seems to form an inner zone. In particular, he has discussed the significance of the diurnal distribution of auroral frequency at various northern hemisphere stations. At these stations inside the auroral zone there are two maxima in the diurnal frequency curve, commonly called the morning and afternoon maxima; similar frequency variations inside the southern zone have been reported by Hatherton and Midwinter[4] and Malville[5]. The auroral observations at Scott Base ($\varphi_m = -79{\cdot}0°$) and Hallett Station ($\varphi_m = -74{\cdot}7°$) have been examined to see if there is any evidence of an inner zone inside the southern auroral zone. The geomagnetic latitudes of these stations are such that the stations lie between the normal zone and the predicted inner zone, and auroræ in both zones should be visible from both stations.

The distribution of all auroral forms visually observed on the quarter-hour during 1957 is shown in Fig. 1 for both stations. The sky has been divided into octants and three zones of elevations 0–20°, 21–50°, and 51–90°, and the presence of any form or part of a form in a segment recorded. The activity is most prominent in the north-east quadrant, which contains the geomagnetic meridian at the stations. In this figure are also shown the directions for all forms observed at Cape Adare and Cape Evans in 1911, a period of sunspot minimum, by the British Antarctic (Terra Nova) Expedition[6]. The distribution shown in Fig. 1 is not inconsistent with the idea of a single zone centred on the geomagnetic pole and of radius 18–20°, with the exception of the rather high activity, particularly at high elevations, in the azimuths 150–200°. This activity is due to the southern extensions of arcs and bands passing through the zenith in a direction roughly north–south. Analysis of the directions of arcs[7] has shown that at Scott Base the most frequent direction of normals to the arcs is 110°, whereas the geomagnetic meridian is 058°. At Hallet Station at low elevations the normals are at 050°, a direction not far removed from the geomagnetic meridian (040°) but at higher elevations they swing round to 070°. The morphology seems to consist of a ray distribution and an arc and band distribution superimposed, but with different axes of symmetry; the isolated rays have a maximum occurrence to the north-north-east.

The directions of the arcs at both Scott Base and Hallett Station agree very well with the isochasms of the southern zone drawn by Gartlein, Nack and Sprague[8], and also with a theoretical zone predicted by Quenby and Webber[9]. The latter authors have considered the effect on cosmic ray trajectories of the non-dipole parts of the field. They have obtained a latitude $\bar{\lambda}$ with respect to a geomagnetic dipole tilted to give the best fit to the horizontal and vertical components of the field at any point. Fig. 2 shows the isopleth for $\bar{\lambda} = -70{\cdot}4°$ which, according to Quenby and Webber, constitutes the zone position at sunspot minimum; also shown are the isochasms of Gartlein, Nack and Sprague and the normals to arcs at Hallett Station and Scott Base. Many attempts both theoretical and empirical have been made to locate the zone of maximum auroral occurrence. (For summary of these attempts see Bond and Jacka[10].) Of these attempts only the theoretical zone of Quenby and Webber and the empirical isochasms of Gartlein, Nack and Sprague are centred in a region corresponding to the directions of minimum activity at Scott Base and Hallett Station.

The distribution of latitudes of only arcs and arc-like bands is shown for Scott Base in Fig. 3. (The highest point of the arc when the latter is first observed is the recorded elevation. The afternoon maximum is at 17 hr. local time and the morning

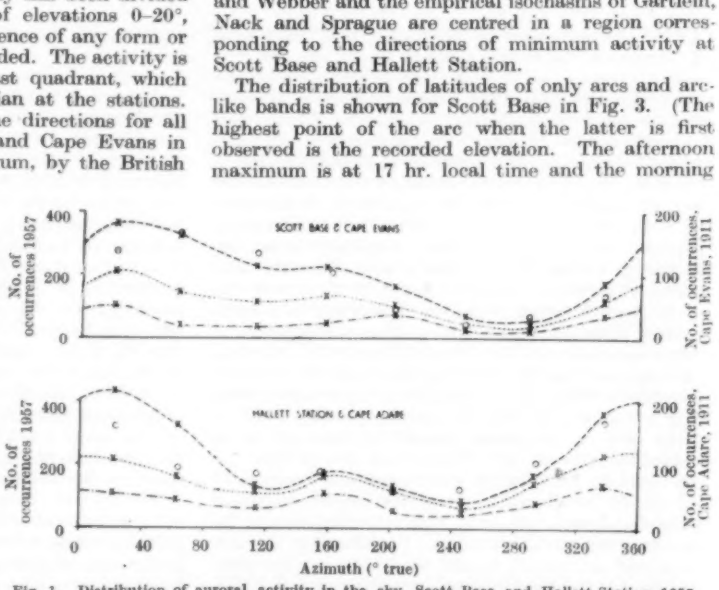

Fig. 1. Distribution of auroral activity in the sky, Scott Base and Hallett Station, 1957. The azimuthal distribution of auroræ at Cape Evans and Cape Adare during 1911 is shown for comparison ○, all auroræ (1911); – – –, 0–20° elevation (1957); · · ·, 21°–50° elevation (1957); – · – · –, 51°–90° elevation (1957)

maximum at 04 hr. local time.) Even allowing for the tendency of observers to class any elevation above 60° as zenithal there is still a high proportion of arcs occurring virtually overhead. The situation at Hallett (not shown) is similar. The main activity is at lower latitudes in the afternoon than during the morning. As the afternoon is the more geomagnetically disturbed period the distribution is consistent with the findings of Hatherton and Midwinter[7] that the latitude of an auroral form is a function of geomagnetic activity at the station. This effect may be enhanced by the slight twilight because the eastern horizon will tend to be lighter during the morning maximum. Otherwise there does not appear to be any radical difference between the distribution of arc latitudes in the two periods considered. Certainly zenithal activity does not seem confined to either the morning or afternoon maximum. Moreover, if the distributions of arc latitudes are corrected for the increasing area of the sky as the elevation interval moves from the zenith to the horizon, the resulting density of auroral arcs shows a pronounced maximum on the outer (northern) side of the zenith at both Scott and Hallett, with secondary maxima in the lower part of the sky. The absolute relationship of inner and outer maxima depends on the manner in which the visibility decreases with distance from the stations. At both stations the extremely rapid decrease in activity as the zenith is crossed (from magnetic north to south) can only be described as remarkable.

Lassen[3] has concluded that at the main zone there is only one maximum, near geomagnetic midnight, in the diurnal variation of auroral frequency and that as the inner zone is approached a second (morning) maximum increases in intensity; this morning maximum becomes the dominant one as the pole is approached. The evidence from Scott Base and Hallett Station is not altogether consistent with this simple picture. In the first place activity is a minimum at geomagnetic midnight, though the afternoon maxima are close to a magnetic midnight, taking the affective pole as the centre of Quenby and Webber's zone: at the South Pole[8] both midnights fall in a period of low activity. Secondly, although the frequency of occurrence of auroræ at Hallett (80 per cent of clear dark hours have auroræ) is much higher than at Scott (27 per cent), suggesting that Hallett is much nearer the main zone, nevertheless the relative development of both maxima is similar at Hallett and Scott[4], and the absolute frequencies at Hallett are much higher than at Scott at both maxima. This could only make sense in Lassen's scheme if Scott Base were well inside the inner zone and Hallett Station just inside the inner zone, as in both cases most auroræ occur to the north and east; thus a polar distance of at least 13° would be indicated for the inner zone.

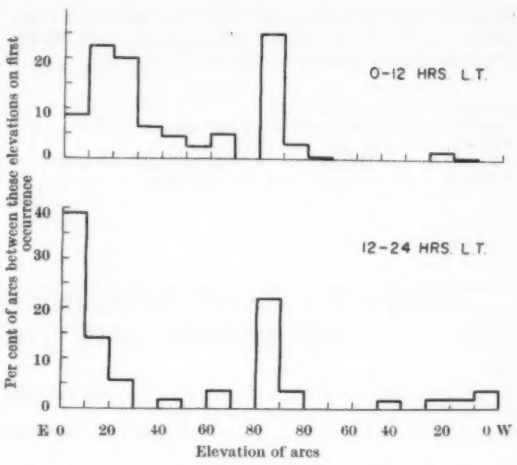

Fig. 3. Distribution of elevations of arcs on first visual observation, Scott Base, 1958. Histograms are shown for 0–12 hr. local time (includes morning maximum) and 12–24 hr. local time (includes afternoon maximum)

These postulates are difficult to accept for several reasons, and they conflict with the proposition that the zenithal auroræ at Scott Base are of the inner zone, although the truth of this proposition is not established. Thirdly, there is the surprising result that the diurnal variation of auroral frequency is not matched by a similar variation in magnetic disturbance at these stations as would be required by Alfvén's theory. The bi-modal diurnal curve of geomagnetic disturbance characteristic of the Stagg[11] transition zone is shown only by Little America of stations examined so far, and this station is near the zone of Quenby and Webber. Singer[12], however, has suggested that it may be possible to produce low-altitude aurora without appreciable geomagnetic effects.

It can be seen from Fig. 2 that the distances of Scott Base and Hallett Station from the Quenby and Webber zone are almost identical; the auroral frequencies at the two stations are very different and more in keeping with their geomagnetic latitudes. Again, the nature and intensities of auroral forms at Scott and Hallett are quite different. The morphological picture is further complicated by the description of the unique auroral behaviour at Dumont d'Urville ($\varphi_m = -75{\cdot}7°$)[13]. Thus the auroral geometry inside the southern zone appears far from simple, and an inner zone cannot be ruled out, although if such a zone is present it probably has a polar distance greater than that predicted by Alfvén. The arc directions and the directions of minimum activity at Scott Base and Hallett Station do appear,

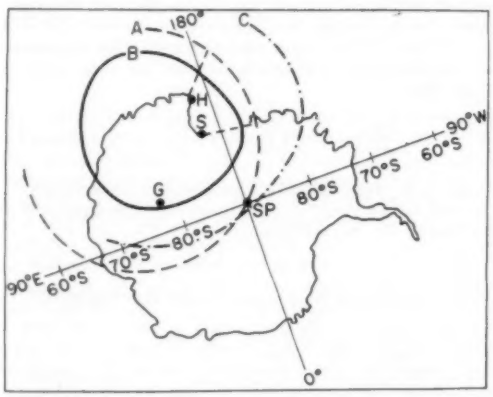

Fig. 2. *A*, theoretically computed zone after Quenby and Webber; *B,C*, auroral 'zones' for small and medium auroræ respectively (after Gartlein, Nack and Sprague); *G*, geomagnetic pole; *H*, Hallett Station; *S*, Scott Base; *SP*, south geographical pole. The dashed lines show the most frequent directions of arcs at Hallett Station and Scott Base

however, to be consistent with an effective zone centre between the south geomagnetic and south magnetic poles and nearer the latter.

[1] Alfvén, H., *Tellus.*, **7**, 50 (1955).
[2] Alfvén, H., "Cosmical Electrodynamics" (Oxford, 1950).
[3] Lassen, K., *Nature*, **184**, 1375 (1959).
[4] Hatherton, T., and Midwinter, G. G., *Nature*, **184**, 889 (1959).
[5] Malville, J. M., *J. Geophys. Res.*, **64**, 1389 (1959).
[6] Wright, C. S., "Observations of the Aurora", British Antarctic (Terra Nova) Expedition, 1910–1913 (1921).
[7] Hatherton, T., and Midwinter, G. G., *J. Geophys. Res.* (in the press).
[8] Gartlein, C., Nack, B., and Sprague, G., *Trans. Amer. Geophys. Union*, **40**, 288 (1959).
[9] Quenby, J. J., and Webber, W. R., *Phil. Mag.*, **4**, 90 (1959).
[10] Bond, F. R., and Jacka, F., Proc. Antarctic Symp., Buenos Aires (1959).
[11] Stagg, J. M., *Proc. Roy. Soc.*, A, **149**, 298 (1935).
[12] Singer, S. F., *Trans. Amer. Geophys. Union*, **38**, 175 (1957).
[13] Weill, G., *C.R. Acad. Sci., Paris*, **246**, 2925 (1958).

PULSATILE FLOW THROUGH TAPERED DISTENSIBLE VESSELS, REFLEXIONS, AND THE HOSIE PHENOMENON*

By ROBERT L. EVANS

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THE effect of vessel taper on pulsatile flows of liquid through distensible tubes has not previously been worked out and is presented here for flows in which viscosity effects are negligible. Such flows have large values of the dimensionless parameter $\alpha = R(2\pi f/\nu)^{1/2}$, in which R is vessel radius, f is frequency, and ν is kinematic viscosity. This parameter was introduced by Valembois[1] and used by Womersley[2], whose results can be used to show that as α is increased the amplitude of his laminar viscosity term approaches $2/\alpha$ times the amplitude of the pressure gradient and is less than 10 per cent of the latter whenever $\alpha > 2{\cdot}5$ (or $R > 1{\cdot}8$ mm. if $f = 1$ sec.$^{-1}$ and $\nu = \pi/100$ cm.^{2} sec.$^{-1}$). Therefore, the flows to which the present theory should be applicable include flows of blood in the larger arteries of mammals.

For the case of horizontal flows (which thus involve no net gravitational effects) the equation of motion in the x or longitudinal direction is:

$$-\frac{\partial p}{\partial x} = \rho\left(\frac{\partial v}{\partial t} + v\,\frac{\partial v}{\partial x}\right) \qquad (1)$$

if p represents pressure, v represents the cross-sectionally averaged velocity, ρ represents density, and t represents time. The additional condition for conservation of a practically incompressible liquid in a vessel of constant length is:

$$\frac{\partial(Av)}{\partial x} = -\frac{\partial A}{\partial t} \quad \text{or} \quad -A\,\frac{\partial v}{\partial x} = \frac{\partial A}{\partial t} + v\,\frac{\partial A}{\partial x} \qquad (2)$$

if A represents the total cross-section at distance x. If also the vessel distensibility is such that constant $k_e = \dfrac{\partial A}{\partial p}\Big/A$, and if the inherent vessel taper is given by constant $A_1 = \dfrac{\partial A}{\partial p}\Big/A$ (whenever the pressure p is constant), then equation (2) becomes:

$$-\frac{\partial v}{\partial x} = A_1 v + k_e\left(\frac{\partial p}{\partial t} + v\,\frac{\partial p}{\partial x}\right) \qquad (3)$$

For the case of waves travelling in only one direction through an untapered tube $A_1 = 0$, and it has been shown that if $c_0 = 1/\sqrt{k_e\rho}$ then $(\Delta p) = \rho c_0\,(\Delta v)$ gives the relation between changes of pressure and changes of velocity[3]. If waves are travelling in both directions (as with both propagation and reflexion) it is necessary to use equation (1) above to relate changes of pressure to changes of velocity[4]. It can also be shown that in the untapered case one can use the solutions of the linear parts of equations (1) and (3) in approximations of the non-linear terms of those equations (which amounts to assuming that v is small relative to c_0 and that v can be treated as a momentary constant) to establish that:

$$\text{propagation velocity } c = c_0 \pm v$$

approximately, if v is the velocity in the positive x-direction and if the plus sign refers to a forward propagated wave and the minus sign refers to a backward propagated wave[4].

To consider pulsatile flows in a tapered and distensible tube one can obtain a fairly simple first approximation by assuming that A_1 is a non-zero constant (as it would be for an exponentially horn-shaped section of vessel) and by neglecting the non-linear terms, or the last terms of equations (1) and (3). When this is done and the resulting equations are combined in a single equation for v or p, it is found that:

$$\frac{\partial^2 y}{\partial t^2} - c_0^2\left(\frac{\partial^2 y}{\partial x^2} + A_1\,\frac{\partial y}{\partial x}\right) = 0 \qquad (4)$$

where y represents either pressure p or velocity v. An eventually fruitful treatment of this equation is the use of the substitution:

$$y = u \cdot e^{-A_1 x/2}$$

which transforms equation (4) into the equation:

$$\frac{\partial^2 u}{\partial t^2} - c_0^2\left(\frac{\partial^2 u}{\partial x^2} - \frac{A_1^2}{4}\,u\right) = 0$$

and which can be followed by the rotatory changes of independent variables:

$$r = (t - x/c)\,/\,\sqrt{2} \text{ and } s = (t + x/c)\,/\,\sqrt{2}$$

which in turn lead to the equation:

* The words "Hosie Phenomenon" are used here to designate the marked smallness of certain observed propagation velocities in comparison with analogous ones that can be related, through classical equations like the Bramwell–Hill equation, to the associated vessel distensibility (even when the viscous and non-linear terms are also considered). However, Dr. Hosie objects to this use of the word "phenomenon".

$$\left(1-\frac{c_0^2}{c^2}\right)\left(\frac{\partial^2 u}{\partial s^2}+\frac{\partial^2 u}{\partial r^2}\right)+2\left(1+\frac{c_0^2}{c^2}\right)\frac{\partial^2 u}{\partial r\,\partial s}+2\left(\frac{c_0 A_1}{2}\right)^2 u=0 \quad (5)$$

This equation is satisfied if simultaneously:

$$\frac{\partial u}{\partial s}=0 \text{ and } \left(1-\frac{c_0^2}{c^2}\right)\frac{\partial^2 u}{\partial r^2}+2\left(\frac{c_0 A_1}{2}\right)^2 u=0 \quad (6)$$

which imply physical transmission by either of two possible modes.

Mode 1, or sinusoidal mode (in which $c > c_0$ and $|\beta| < 1$):

One solution of equations (6) is:

$$u = E \sin \sqrt{2}\,\omega\,(r+\varphi) \quad (7)$$

where $\omega = 2\pi f$, $c = c_0/\sqrt{1-\beta^2}$, and $\beta = c_0 A_1/4\pi f$. This solution (equation (7)) is a sinusoidal wave of frequency f sec.$^{-1}$ and travelling in the positive x-direction with propagation velocity c. It shows that the dimensionless parameter:

$$\beta = c_0 A_1/4\pi f \quad (8)$$

appropriately describes a pulsatile flow through a tapered vessel. This parameter is assumed to be less than unity in getting the solution in equation (7).

A second solution representing a reflected wave in the tapered distensible vessel can be obtained from equation (5) in a similar manner by reversing the roles of the variables r and s. The final solution for pressure or velocity is thus:

$$y = e^{-A_1 x/2}\,[E \sin \omega\,(t - x/c + \varphi_1) + F \sin \omega\,(t + x/c + \varphi_2)]$$

in which ω and c have their previously defined values. In this solution E expresses the amplitude of the propagated wave and F expresses the reflected amplitude of the same frequency. The phase angles φ_1 and φ_2 have different values for associated pressure and velocity waves and are thus such that if $\gamma = \arcsin \beta$ (principal value) then the complete solution for frequency $\omega/2$ is:

$$v = e^{-A_1 x/2}\left[E \sin \omega\left(t-\frac{x}{c}\right)+F \sin \omega\left(t+\frac{x}{c}\right)\right]$$

and $$p = \rho c_0 e^{-A_1 x/2}\left[E \sin \omega\left(t-\frac{x}{c}+\frac{\gamma}{\omega}\right)-F \sin \omega\left(t+\frac{x}{c}-\frac{\gamma}{\omega}\right)\right] \quad (9)$$

assuming that the origins of time and distance are appropriately chosen. These functions are such that the taper coefficient A_1, parameter β, and phase angle γ will all have the same sign and be positive or negative together. Hence the observer at a given site along a section of positive taper will see a forward propagated pressure wave leading its associated velocity wave and a retrograde-propagated pressure wave following its associated velocity wave, and both by the $\gamma/2\pi$th part of one cycle.

Two noteworthy comments are: first, that the attenuation factor $e^{-A_1 x/2}$ has half of the taper coefficient A_1 as the coefficient in the exponent—and not A_1 itself; and secondly, that in a tapered section the propagation velocity $c_0/\sqrt{1-\beta^2}$ depends on both the frequency and the taper, while that in an untapered section is independent of the frequency.

Mode 2, or exponential mode (in which $c < c_0$):

Another possible solution of equations (6) is of the form:

$$v = e^{-A_1 x/2}\,[E\,e^{\omega(t-x/c)} + F\,e^{-\omega(t-x/c)}] \text{ and}$$

$$p = \rho c_0 e^{-A_1 x/2}\left[\frac{E\,e^{\omega(t-x/c)}}{\beta+\sqrt{1+\beta^2}}+\frac{F\,e^{-\omega(t-x/c)}}{\sqrt{1+\beta^2}-\beta}\right] \quad (10)$$

where $c = c_0/\sqrt{1+\beta^2}$ and $\beta = A_1 c_0/2\omega$ as before. Equations (10) refer to a forward-propagated wave, as do the E-containing terms of equations (9), while similar expressions containing $(t + x/c)$ instead of $(t - x/c)$ can be derived for a backward-propagated wave. The solutions of this mode are sums of exponential functions and thus can only approximate to sinusoidal waves. They connote a modified physical meaning of ω—namely, the reciprocal of a relaxation time, and they can exist for any values of β, while the sinusoidal mode 1 can exist only where the magnitude of parameter β is less than unity, or $|\beta| < 1$. Arterial pulse waves are shown later to indicate that the sinusoidal mode is present wherever the parameter β is sufficiently small.

With the above solutions one can show that pulses of either mode can give rise to a reflected wave at any corner along the vessel where β (and thus also the taper A_1) has a discontinuity. Since the reflexion at a slight corner is proportional to the associated discontinuity of β, there should be no reflexion in a vessel system the taper of which varies continuously. This zero lower bound for reflexions can be compared with Womersley's estimate of about 10 per cent reflexion in two particular cases assumed to have sudden changes of cross-sectional area corresponding to two corners (inside and outside) at a single distance x in each particular case (ref. 2, Sect. VII).

The Hosie Phenomenon. Hosie's work on the *in vivo* stresses and strains in the thoracic aorta of the anæsthetized dog provides very good, and perhaps the only available, data for simultaneous estimates of the propagation velocity c (measured at or near the foot of the wave) and of the propagation velocity c_0 that can be calculated from measurements of the distensibility k_0 (ref. 5). He found that the c_0 calculated for existing pressures was about 50 per cent greater than the measured c. Only simultaneous measurements like Hosie's did and could reveal this marked discrepancy, which is therefore called the 'Hosie phenomenon'. It is unexplained by factors other than taper, but since the proximal thoracic aorta is one of the most tapered parts of the arterial tree it is reasonable to attribute the Hosie phenomenon to a pulsatile flow of mode 2 and a taper parameter $\beta < -1$, for which equation (10) gives:

$$c_0 = c\sqrt{1+\beta^2}$$

or c_0 at least 41 per cent greater than c. This explanation implies that the Hosie phenomenon occurs in all regions of sufficient cross-sectional taper.

The detailed study of reflexions is too long for this article, and a full study of transitions between modes 1 and 2 is yet to be made.

This work was supported by a U.S. Public Health Service Grant *H*3680, which is gratefully acknowledged.

[1] Valembois, J., "Contribution à l'Etude de la Mésure des Pressions Variables", 39 (Hermann and Co., Paris, 1948).
[2] Womersley, John R., "An Elastic Tube Theory of Pulse Transmission and Oscillatory Flow in Mammalian Arteries", Wright Air Development Ctr. Tech. Rep. *TR* 56–614 (January 1958).
[3] Evans, Robert L., *Nature*, **181**, 1471 (1958).
[4] Evans, Robert L., *J. App. Physiol.*, **15** (No. 2) (1960).
[5] Hosie, Kenneth F., University of Minnesota Ph.D. thesis, 144 (September 1957).

REGENERATION OF VISUAL PIGMENTS FROM THEIR LOW-TEMPERATURE PHOTOPRODUCTS

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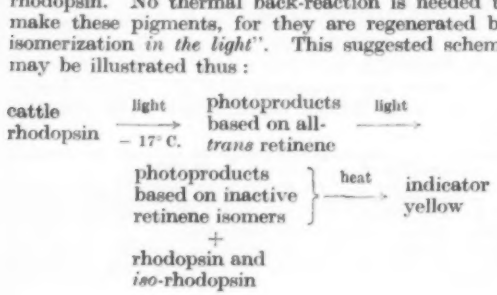
FOR most practical purposes a thermally stable compound known as indicator yellow[1] is the ultimate product in the bleaching of visual pigments at room temperature. However, irradiated solutions of frog rhodopsin at 3° C.[1,2] and of cattle rhodopsin mixed with glycerol at − 17° C.[3,5] have been shown to contain fairly stable orange photoproducts with maxima situated roughly 15–20 mμ below those of the corresponding parent pigments. Other photoproducts, but with maxima displaced by only 5 mμ towards the blue, have been described for water–glycerol solutions of frog rhodopsin at − 73° C.[4] and for cattle rhodopsin at − 45° C.[5].

Wald and his colleagues, using cattle rhodopsin[3,5], and Collins and Morton, using frog, rat and ox rhodopsins[6], have shown that warming these low-temperature products causes loss of part of the light-absorbing material, but that the remainder is thermally stable and photosensitive. This residue, with its $\lambda_{max.}$ at about 8 mμ below the parent rhodopsin, was originally called '*iso*-rhodopsin'[6] or 'regenerated rhodopsin'[5]. Later, Hubbard and Wald[7] claimed, but did not demonstrate, that this material was a mixture of the parent rhodopsin and the corresponding true *iso*-rhodopsin (produced by substituting *iso*-retinene$_1$ *a* for neo-retinene$_1$ *b* in the rhodopsin molecule).

Both Wald and Collins and Morton originally believed that the residual photosensitive chromogen was generated from the low-temperature product during the warming process. More recently, however, Kropf and Hubbard[3] have produced evidence to support the notion that the low-temperature product itself is a mixture of 'regenerated rhodopsin' and a chromophore or chromophores unstable at room temperature. According to this hypothesis, warming merely converts the latter to indicator yellow and unmasks the 'regenerated rhodopsin'.

Kropf and Hubbard have explained the low-temperature work as follows: light isomerizes the neo-retinene *b* chromophore of rhodopsin to form photoproducts based on all-trans retinene. "Light can then go on to isomerize the chromophore . . ." [of these photoproducts] ". . . to a steady state mixture of geometrical isomers, all still attached to opsin. The neo-*b* component of this mixture is itself rhodopsin; the *iso-a* component is itself *iso*-rhodopsin. No thermal back-reaction is needed to make these pigments, for they are regenerated by isomerization *in the light*". This suggested scheme may be illustrated thus:



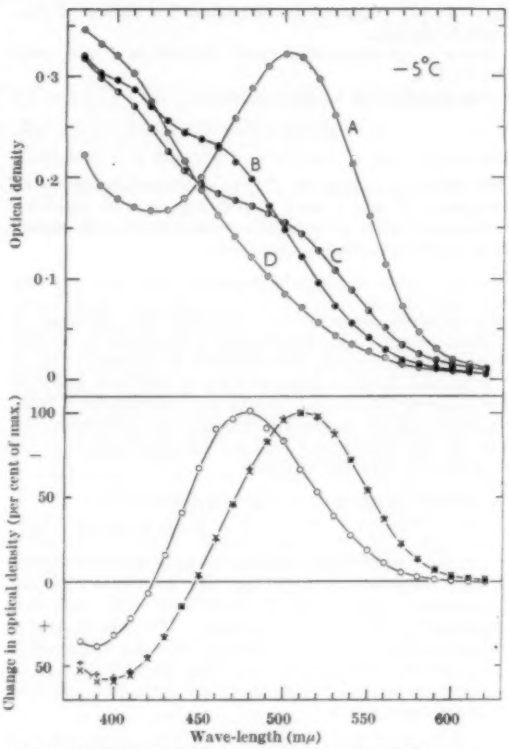


Fig. 1. Regeneration of frog rhodopsin by warming its low-temperature photoproduct. (All density spectra measured at −5° C.; solution in 2 per cent aqueous digitonin diluted with an equal volume of glycerol; pH = 5·8)
Upper half of figure: curve *A* is the initial density spectrum; curve *B* after 1 hr. λ550 mμ irradiation at −5° C.; curve *C* after warming to room temperature and re-cooling; curve *D* after exposure to white light at room temperature and re-cooling. Lower half of figure: ○, difference spectrum for the low-temperature photoproduct (*D* minus *B*); +, difference spectrum for regenerated rhodopsin (*D* minus *C*); ×, difference spectrum for parent rhodopsin (*D* minus *A*)

No mention has been made here of lumi- and meta-rhodopsin, names devised by Wald *et al.*, originally for the low-temperature photoproducts of cattle rhodopsin, the similar flash photoproducts of cattle rhodopsin in dry gelatin films at room temperature and for the *p*H-sensitive photoproduct of squid rhodopsin below + 15° C.[5,8]. Recently, these terms have been refined in order to bring them into line with the hypothesis of bleaching outlined above. In view of the results considered here and others to be published elsewhere, I think that this nomenclature is unsuitable for describing the different and very complicated reactions which occur when rhodopsins are exposed to light under a wide variety of conditions. Until there is a better understanding of these reactions the use of a terminology implying specific mechanisms should in any event be avoided.

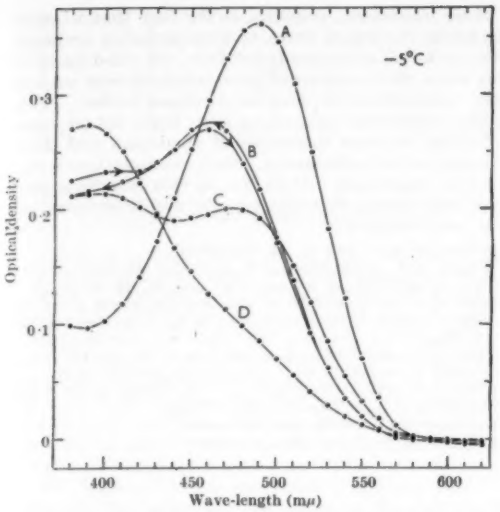


Fig. 2. Regeneration of conger eel rhodopsin by warming its low-temperature photoproduct. (All density spectra at −5° C.; solution in 2 per cent digitonin diluted with an equal volume of glycerol; pH = 5·8)
Curve *A* is the initial density spectrum; curve *B* after 1½ hr. λ550 mμ irradiation at −5° C.; curve *C* after warming to room temperature and re-cooling; curve *D* after exposure to white light at room temperature and re-cooling

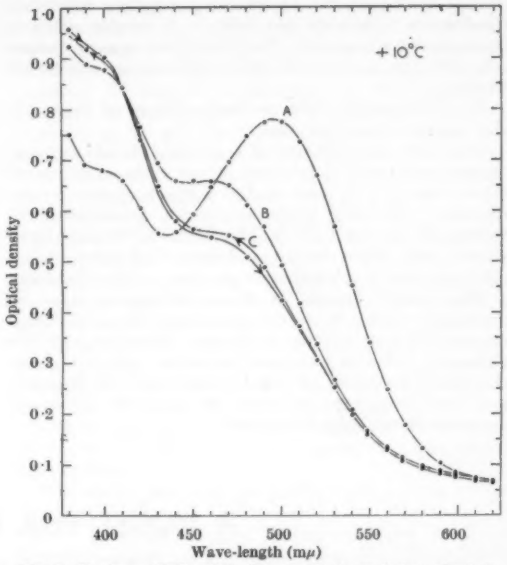


Fig. 3. Photosensitivity of the low-temperature photoproduct of rhesus rhodopsin. (All density spectra at +10° C.; solution in 2 per cent aqueous digitonin; no glycerol added; pH = 6·5). Curve *A* is the initial density spectrum; curve *B* after 2 hr. λ580 mμ irradiation at +10° C.; curve *C* after 35 min. λ500 mμ irradiation at +10° C.

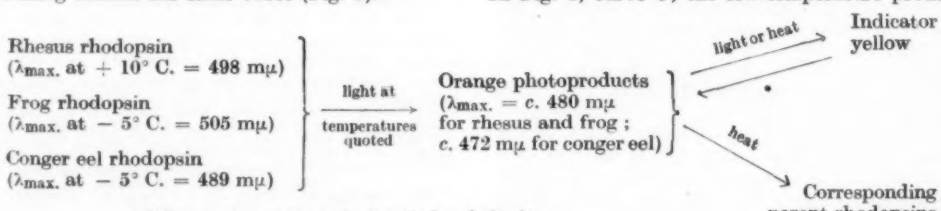
In the present work results of low-temperature studies on visual pigments extracted from the whole, acid-washed retinæ of rhesus, frog and conger eel are considered. Some of these results are in marked disagreement with the picture of the bleaching process sketched by Kropf and Hubbard.

Prolonged monochromatic irradiation of frog and conger eel rhodopsins at − 5° C. in glycerol/aqueous digitonin and rhesus rhodopsin at + 10° C. in aqueous digitonin generates a mixture of indicator yellow and a stable product with $\lambda_{max.}$ displaced about 20 mμ towards the blue. Thus, in Fig. 1 curve *A* represents the density spectrum of frog rhodopsin cooled to − 5° C. and curve *B* the density spectrum of the product of prolonged exposure to λ550 mμ light. At this temperature the photoproduct is stable (measurements at 20 mμ intervals from 380 to 620 mμ interlacing with return measurements from 610 to 390 mμ give a check of stability[9]). Warming to room temperature and re-cooling causes a bodily shift of the extinction curve towards longer wave-lengths, as shown by curve C. This thermally stable material, when irradiated at + 25° C., gives a difference spectrum identical with the difference spectrum of the parent frog rhodopsin as shown in the lower half of Fig. 1. The rhodopsins of conger eel ($\lambda_{max.}$ = 487 mμ at + 20° C.[10]) and of rhesus ($\lambda_{max.}$ = 497 mμ at + 37° C.[11]) similarly yield photoproducts which on warming exhibit the same effect (Fig. 2).

These experiments demonstrate beyond doubt that the bulk of the parent rhodopsin is generated from part of the low-temperature product *during the warming process*, and is not merely 'unmasked' by destruction of a thermolabile chromophore, as Kropf and Hubbard have found. Were the latter the case, the warming would produce an overall fall in density characteristic of the thermolabile component—the observed rise in density at long wave-lengths could not occur.

In the case of rhesus rhodopsin the low-temperature product is photosensitive. Thus, in Fig. 3, curve *B* shows the product of prolonged λ580 mμ irradiation of rhesus rhodopsin at + 10° C., and curve *C* after irradiation of this product with λ500 mμ light. Loss in density occurred beyond 410 mμ, maximal at 465 mμ (between 465 and 480 mμ in other similar experiments). Below 410 mμ there was a rise in density showing that the product of irradiation was probably indicator yellow. Photosensitivity of the primary photoproduct probably also accounts for the presence of indicator yellow in solutions of frog and conger eel rhodopsins irradiated at − 15° C.

Not only is the low-temperature photoproduct capable of bleaching to indicator yellow when exposed to light, but in some cases, when afterwards left in darkness, the reverse process can also occur. In Fig. 3, curve *C*, the low-temperature product of



• Reverse process apparently absent in frog rhodopsin.

Fig. 4

rhesus rhodopsin can be seen regenerating following irradiation with λ500 mμ light. A similar effect is illustrated by curve *B*, Fig. 2, for the case of conger eel, although no trace of this regeneration was found in frog.

Fig. 4 illustrates the probable chain of events in the experiments described.

It is not intended that this scheme should be anything more than a summary of some observations on three visual pigments under a specific set of conditions. At this stage it is not permissible to generalize, and indeed Wald and his colleagues have shown that when other conditions and other visual pigments are used different results can be obtained.

The possible cause of these differences may be connected with *p*H and temperature. Thus, the frog, conger eel and rhesus rhodopsin solutions had *p*H values of 0·9 and 1·6 units below the *p*H 7·4 cattle rhodopsin solutions of Wald, Durell and St. George[5], and the temperatures were 12° and 27° C. above those of Kropf and Hubbard[3].

More important, perhaps, is the fact that whereas the cattle rhodopsin extracts were probably prepared from isolated, alum-hardened rods, the rhodopsins of this work were extracted from whole retinæ washed only with *p*H 4·6 phosphate–citrate buffer. This milder extraction procedure may leave intact combinations between molecules of rhodopsin and those of other retinal substances. Such combinations could take an important role in the *in vivo* bleaching process, and could affect the type of result obtained at low temperatures.

[1] Lythgoe, R. J., *J. Physiol.*, **89**, 331 (1937).
[2] Lythgoe, R. J., and Quilliam, J. P., *J. Physiol.*, **94**, 399 (1938).
[3] Kropf, A., and Hubbard, R., *Ann. N.Y. Acad. Sci.*, **74**, 266 (1958).
[4] Broda, E. E., and Goodeve, C. F., *Proc. Roy. Soc.*, A, **179**, 151 (1941).
[5] Wald, G., Durell, J., and St. George, R. C. C., *Science*, **111**, 179 (1950).
[6] Collins, F. D., and Morton, R. A., *Biochem. J.*, **47**, 18 (1950).
[7] Hubbard, R., and Wald, G., *J. Gen. Physiol.*, **36**, 269 (1952).
[8] Hubbard, R., and St. George, R. C. C., *J. Gen. Physiol.*, **41**, 501 (1958).
[9] Dartnall, H. J. A., *J. Physiol.*, **116**, 257 (1952).
[10] Walker, M. A., *J. Physiol.*, **133**, 56*P* (1956).
[11] Bridges, C. D. B., *Nature*, **184**, 1727 (1959).

A MODEL FOR MORPHOGENESIS

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THIS article describes a model which imitates certain aspects of morphogenesis and maintenance in a developing embryo. The model consists of a number of artificial 'cells' (constructed from radio parts) which can stimulate and inhibit one another by means of connexions which are made through a switchboard. The pattern of activity generated, representing the growth pattern, was found to bear a remarkable resemblance to certain aspects of morphogenesis.

It is almost self-evident that the development of an embryo from a fertilized egg involves communication and control processes between the cells of the organism. Information must pass from cell to cell, enabling the organism to compare what is with what ought to be, and to correct the difference. The nature of this information transfer is unknown; but that it must occur, and to some extent by direct contact, is shown by many experiments, for example, those of Driesch[1]: when the blastomeres after the first one or two cleavages are separated, it is possible to obtain twins or quadruplets of smaller size than normal instead of half- or quarter-embryos; as if the blastomeres 'knew' what is the condition of the neighbouring cells. Also, Grobstein and Zwilling[2] concluded from transplantation experiments of minced embryonic tissues that, for further organized growth to occur, the preservation of a certain amount of cell-to-cell relationship was necessary; this would not be expected with a purely humoural mechanism. The absence of firm and permanent connexions between cancer cells, in contrast to those of normal tissue[3,4] may be a factor in their uncontrolled growth.

In order to see what would be the result of the communication between cells connected together in a particular network, models were made. Each model 'cell' was constructed as in Fig. 1. It had the property of receiving an electrical pulse from any similar 'cell' connected to its input, and after a short delay passing out a pulse to any other 'cells' connected to its output. The arrival of a pulse was indicated by the lighting of a lamp attached to the 'cell'. If, for example, four such 'cells' were connected in a circular path (Fig. 2*a*) the pulse circulated indefinitely. Interesting patterns are generated when they are connected in other ways. In the symmetrical circuit in Fig. 2*b*, in which each 'cell' has two input and two output leads, the surprising result of an unsymmetrical pattern of pulses was obtained: the final result, after starting a pulse anywhere in the system, was that the lamp of one 'cell' pulsated while that of the others remained on. This shows how it is possible to obtain an asymmetry or head-tail polarity among identical units or 'cells' connected symmetrically.

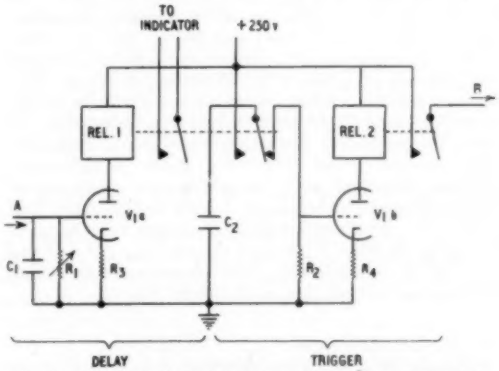

Fig. 1. A positive pulse arriving at input *A* charges C_1, reduces the negative potential on the grid of V_1a and closes *REL* 1. The delay time is governed by R_1; when the grid of V_1a becomes negative once more, *REL* 1 switches C_2, which has been charged during the time delay, across the low resistance R_2. This brings the grid of V_1b positive for about a quarter of a second, causing *REL* 2 to close for the same duration, thereby sending a pulse via output *B* to the next unit or units
$R_1 = 100\ K\Omega$, $R_2 = 1\ K\Omega$, $R_3 = 6{\cdot}8\ K\Omega$, $R_4 = 6{\cdot}8\ K\Omega$;
$C_1 = 8\ \mu F$, $C_2 = 2\ \mu F$;
V_1a,b is ECC 82; *RELS* 1 and 2 = $3{\cdot}5\ K\Omega$

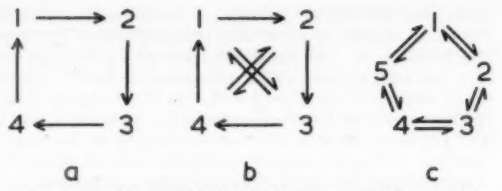

Fig. 2. Circuits showing some of the ways in which the 'cells' (represented by numbers) were connected together

How an embryo develops a head end, from cells which are initially all equivalent, has always been a puzzle. That the cells are initially equivalent has been shown by numerous interchange experiments, as well as those of Driesch[1] (for reviews, see Waddington[5] and Needham[6]). In our system, the true equilibrium position for the circuit shown in Fig. 2*b* is one of polarity; this is also the case when the connexions between the cells are as in Fig. 2*c* with any odd number of cells above three; and with various alternative ways of constructing the unit 'cell'.

The reason for the asymmetry appears to be as follows: the mounting excitation in the system due to the repeated passage of pulses tends to drive each 'cell' into a pulsating state; however, the first 'cell' to reach this state inhibits the others from pulsating (briefly, each pulsation in one 'cell' charges the condenser of the other 'cells" and so prevents their pulsating). It is not difficult to see the analogies for this in real cells in terms of chemical or electrical capacities.

While the final state in our system is one of polarity, the system has a symmetry of another kind: if the cell which is pulsating is prevented from doing so by holding down its relay, another cell starts pulsating. If in turn its relay is held, another one pulsates, and so on until all have been used up. Thus the axis of the polarity can be 'chased' around the system. This behaviour is, in a crude way, reminiscent of brain activity: surgeons have found that it is not possible to remove memories by excising parts of the brain; when a part is inactivated or excised, the remainder functions as a whole. There does seem to be a formal analogy between the behaviour of the brain (with respect to its activity) and of the embryo (with respect to its growth); one wonders whether controlled growth, at least in the early embryo, might not be a result of a kind of 'brain-like' interaction between all the cells of the embryo.

Many other properties of our system, with larger numbers of cells and with other ways of connecting them together, are reminiscent of the properties of growing embryos: for example, the patterns are in general homeostated—a disturbance producing only a temporary change in the pattern. Discrete differences (such as 'on' or 'off') appear between neighbouring cells, in contrast to the continuous differences which would be expected on a purely humoural mechanism of growth control.

A fuller account will be published in the Proceedings of the Second International Congress of Cybernetics, held in Namur, 1958.

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[1] Driesch, H., *Z. wiss. Zool.*, **53**, 60 (1891).
[2] Grobstein, C., and Zwilling, E., *J. Exp. Zool.*, **122**, 259 (1953). See also Grobstein, C., *ibid.*, **120**, 437 (1952).
[3] Coman, D. R., *Cancer Res.*, **4**, 625 (1944). See also, for plant tumours, White, P. R., *Quart. Rev. Biol.*, **26**, 1 (1951).
[4] Ambrose, E. J., James, A. M., and Lowick, J. B., *Nature*, **177**, 576 (1956). See also Abercrombie, M., and Ambrose, E. J., *Exp. Cell. Res.*, **15**, 332 (1958).
[5] Waddington, C. H., "Principles of Embryology" (Allen and Unwin, London, 1956).
[6] Needham, J., "Biochemistry and Morphogenesis" (Cambridge University Press, 1942).

ACTIVITY IN RELATION TO *p*H OF MYOSIN-5′-NUCLEOTIDASE

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THE relative rates of hydrolysis of the nucleotide substrates of myosin vary widely under the different conditions imposed by either changes of temperature or the presence or absence of modifiers. Thus inosine triphosphate is hydrolysed much more rapidly than adenosine triphosphate in the presence of calcium ions at 25° C., but the latter is hydrolysed more rapidly than the former at 0° C., or in the presence of *p*-chloromercuribenzoate or 2,4-dinitrophenol at either temperature. These facts have never been adequately explained in terms of the structure of the enzymatic site of myosin. In an earlier paper[1], a theory to account for them, based on the reported aggregation of myosin at room temperature and the supposed effect of such aggregation on enzyme activity, was proposed; but subsequent attempts to confirm this from light-scattering data have failed. A different approach, based on the variation of activity with *p*H of myosin under different conditions, has yielded valuable information, which allows some conclusions to be drawn with regard to the nature of the enzymatic site.

All the experiments reported here were done with myosin *A* prepared from rabbit muscle by the method of Szent-Györgyi[2]. The preparation as finally purified showed no change in light scattering on the addition of adenosine triphosphate. Adequate stability over the desired *p*H range of 5·3–9·8 was achieved by using a mixed *tris* (hydroxymethyl) aminomethane histidine buffer of the following composition: *tris*, 0·05 *M*; histidine, 0·05 *M*; potassium chloride, 0·08 *M*; calcium chloride, 0·001 *M*. In one experiment the concentration of potassium chloride was increased to 0·5 *M*. Activities, which were measured at either 25° C. or 0° C., were based on a series of not less than four determinations of phosphate evolved with time, from which zero-order velocities of hydrolysis of the nucleotide substrates were determined. Substrates were present initially at a concentration of 0·001 *M*, and not more than 15

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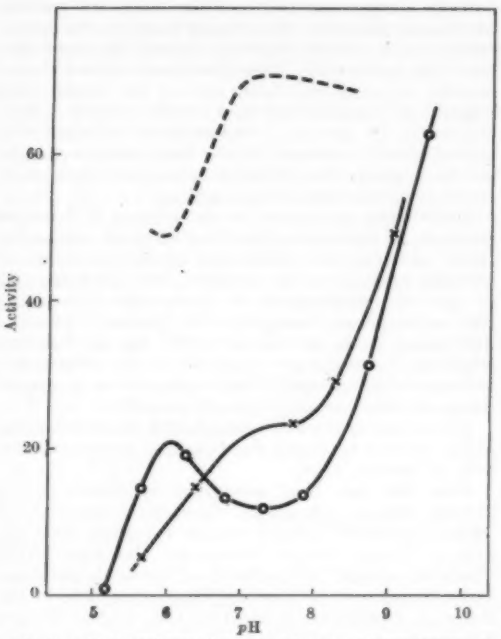


Fig. 1. *p*H dependence of myosin adenosine triphosphatase at 25° C. ○——○, untreated myosin; ×——×, myosin treated with *p*-chloromercuribenzoate. The upper curve represents the difference between the curves for treated and untreated myosin. Activity is expressed in μmoles phosphorus evolved per gm. myosin per sec.

per cent was hydrolysed during the course of an experiment.

In experiments involving myosin treated with *p*-chloromercuribenzoate, the latter was added to the concentrated myosin in an amount sufficient to produce almost maximum activation of the adenosine triphosphatase at 25° C. (*c.* 3 moles per 10^5 gm. myosin). The mixture of myosin and *p*-chloromercuribenzoate was incubated at 25° C. for 30 sec. and then transferred to a refrigerator at 5° C. for several days. It was either warmed or cooled to the appropriate temperature before being added to the buffer-substrate mixture to start the activity run. Under these conditions, the amount of phosphate evolved from the substrate by the myosin treated with *p*-chloromercuribenzoate was linear with time[3].

Effect of *p*H on Activity

The form of the *p*H curve of myosin adenosine triphosphatase at 25° C. (Fig. 1) is well known. It passes through a maximum at an acid *p*H (6·1 under the conditions studied in these experiments), and has a steeply rising alkaline arm. It has been shown[4] that the maximum sometimes recorded in the *p*H range 9·5–10·0 is false, the drop in activity at higher *p*H's being due to irreversible denaturation of the enzyme. The same is not true of the maximum at 6·1, although the enzyme is somewhat more susceptible to denaturation at *p*H's below 6·0 than above it. Thus the activity of the enzyme incubated for 15 min. at *p*H 5·5 and then tested at *p*H 6·2 was still 80 per cent of that of the same enzyme incubated for an equivalent time at *p*H 6·2.

The form of the *p*H curve of myosin adenosine triphosphatase at 0° C. (Fig. 2) is completely different from that at 25° C. At the low temperature, the *p*H curve is simple in form and resembles the hydrogen ion titration of a single dissociable group, except that the *p*H-range over which dissociation would appear to be taking place is very wide. It is notable that at a higher concentration of potassium chloride (0·5 *M*) the *p*H curve at 0° C. is truncated (Fig. 2), as though the higher ionic strength, or higher $[K^+]$ or $[Cl^-]$, had removed part of the activating effect at higher *p*H's. It has been demonstrated by numerous workers that the shape of the *p*H curve of myosin adenosine triphosphatase at 25° C. is not altered from that shown in Fig. 1 at higher ionic strengths, except that there may be a slight shift in the position of the acid maximum.

When inosine triphosphate is the substrate, the *p*H dependence of activity at 25° C. shows two rising arms connected by a region (*p*H 7·2–8·4) over which activity varies very little (Fig. 3). The *p*H curve for inosine triphosphatase at 0° C. (Fig. 4) resembles more closely that for adenosine triphosphatase at the same temperature, except that with inosine triphosphate as substrate the curve approximates even more closely to an acid dissociation curve.

When the protein is modified by treatment with *p*-chloromercuribenzoate, the *p*H dependence of the adenosine triphosphatase at 25° C. (shown in Fig. 1) is altered, so that it now resembles the curve for the hydrolysis of inosine triphosphate by the untreated protein. Thus the well-known activation of the enzyme by *p*-chloromercuribenzoate, which has been studied by several workers[1,2,5], is seen to occur only above *p*H 6·5, and to have a maximum effect at *p*H's between 7 and 8. Below *p*H 6·5, *p*-chloromercuribenzoate inhibits myosin adenosine tri-

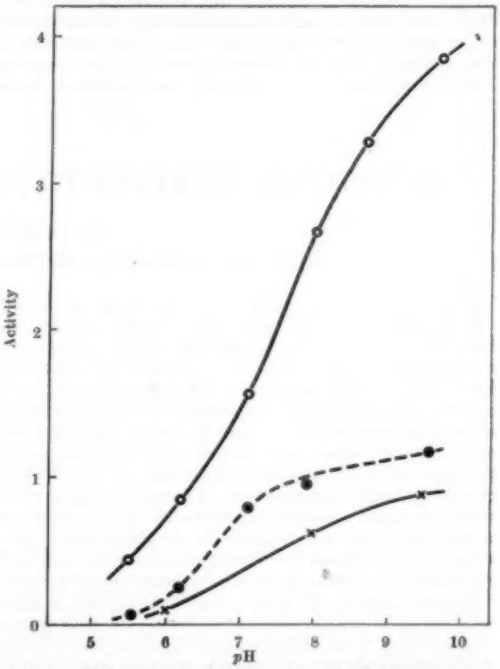


Fig. 2. *p*H dependence of myosin adenosine triphosphatase at 0° C. ○——○, untreated myosin in presence of 0·08 *M* potassium chloride; ● – – – ●, untreated myosin in presence of 0·5 *M* potassium chloride; ×——×, myosin treated with *p*-chloromercuribenzoate in presence of 0·08 *M* potassium chloride

phosphatase. Entirely similar results were obtained when the enzyme was tested in the presence of 5×10^{-3} *M* 2,4-dinitrophenol (Fig. 5), reinforcing earlier evidence[1] that the two reagents produce the same effect on the enzymatic site. An even more pronounced simplification of the *p*H curve of myosin adenosine triphosphatase after treatment with *S*-*B*-aminoethyl*iso*thiuronium, which also ties up sulphydryl groups, was first reported by Morales *et al.*[6], and has recently been studied in greater detail by Morales and Hotta[7].

Treatment with *p*-chloromercuribenzoate caused inhibition of the inosine triphosphatase throughout the entire *p*H-range at 25° C. (Fig. 3). Under these conditions the *p*H curve became simpler in form, with the elimination of the central flattened region between *p*H's 7·2 and 8·4. At 0° C., *p*-chloromercuribenzoate caused severe inhibition of both adenosine triphosphatase (Fig. 2) and inosine triphosphatase (Fig. 4). The very low rates of hydrolysis were measured at three different *p*H's, and the results indicated that the reagent had caused no profound change in the shape of the *p*H curves at the low temperature.

Implications of the *p*H Curves

The kinetic studies of Blum[8] have suggested that the presence of an amino group on the 6 position of the nucleotide substrate of myosin is important in determining both the binding of the substrate to the enzyme and the rate of hydrolysis. At normal temperatures, and *p*H's in the neighbourhood of 7 and 8, at which myosin is usually studied, nucleotides with a 6-amino group, such as adenosine triphosphate, bind very strongly, and the relatively slow rate of hydrolysis is believed to be due to the slow rate of desorption of the products. The acceleration produced by such agents as *p*-chloromercuribenzoate and 2,4-

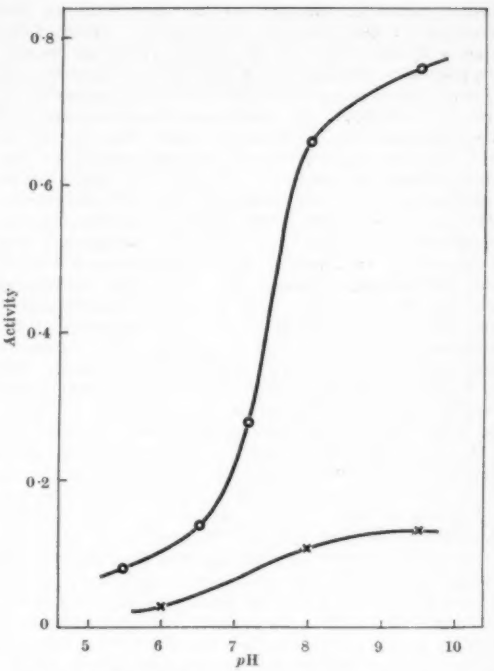


Fig. 4. *p*H dependence of myosin inosine triphosphatase at 0° C. ○——○; untreated myosin; ×——×, myosine treated with *p*-chloromercuribenzoate

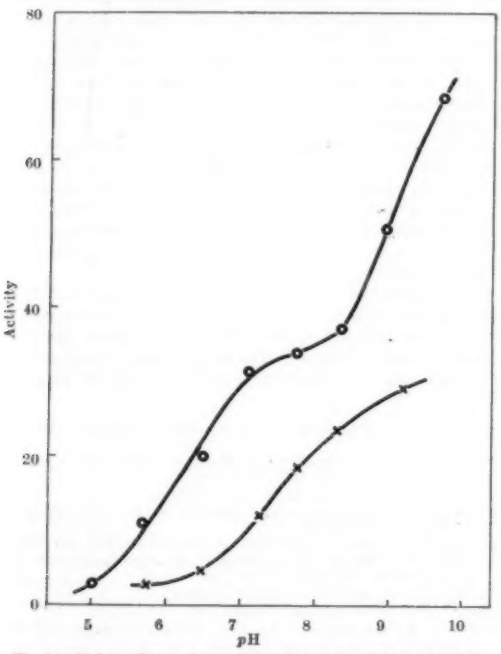


Fig. 3. *p*H dependence of myosin inosine triphosphatase at 25° C. ○——○; untreated myosin; ×——×, myosin treated with *p*-chloromercuribenzoate

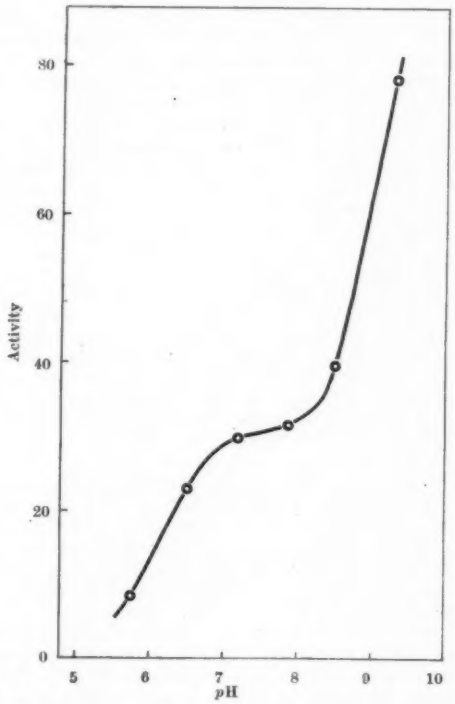


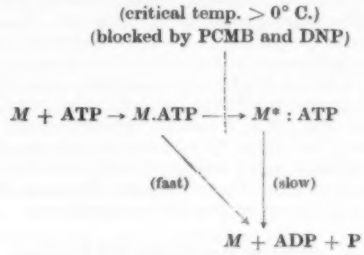
Fig. 5. *p*H dependence of myosin adenosine triphosphatase treated with 2,4-dinitrophenol at 25° C.

dinitrophenol is thought to be the result of the blocking of this 6-amino binding[9–11]. Nucleotides with a hydroxyl in the 6 position, such as inosine triphosphate, which under 'normal' conditions are hydrolysed more rapidly than the 6-amino nucleotides, are only inhibited by *p*-chloromercuribenzoate and 2,4-dinitrophenol. It seems possible that the dip in the *p*H-activity curve of myosin adenosine triphosphatase between *p*H's 6·2 and 8·2 might be an expression of the increasing influence, with decreasing [H^+], of some dissociable group on the enzyme involved in binding to the 6-amino position of the substrate. If this were so, and if this group is blocked by *p*-chloromercuribenzoate, then the difference between the *p*H curve with *p*-chloromercuribenzoate and that without it would be an expression of the dissociation of this particular group. The difference between the *p*H-activity curves of adenosine triphosphatase treated with *p*-chloromercuribenzoate and the untreated enzyme is plotted in Fig. 1. It can be seen that the difference plot does simulate the curve for a single-proton dissociation of a group with a *p*K about 6·5. The decline in the difference plot at *p*H's above 8 is probably not significant, and may be a reflexion of the greater instability and thermal sensitivity of the enzyme treated with *p*-chloromercuribenzoate[10]. It thus appears that the unusual shape of the myosin adenosine triphosphatase-*p*H curve at 25° C. is due to the appearance, with decreasing [H^+], of a negatively charged inhibitory group on the enzyme surface which plays an essential part in the binding of the 6-amino group of the substrate. This group, with a *pK* of 6·5, is presumably not that which itself combines with the 6-amino of adenosine triphosphate, since all the *p*H curves presented in this article were obtained with saturating substrate concentrations, and so reflect dissociations of groups on the enzyme-substrate complex rather than on the free enzyme. Nor is it the —SH to which *p*-chloromercuribenzoate becomes bound, since the *pK* of —SH is much higher.

In the case of the 6-hydroxy nucleotides, the inhibitory binding of the amino substituent does not occur, and there is no sharp drop in the *p*H-activity curve above *p*H 6·2. The *p*H curve, in fact, is very similar to that for the hydrolysis of adenosine triphosphate by the enzyme treated with *p*-chloromercuribenzoate and 2,4-dinitrophenol, and this correspondence, in itself, is very strong evidence in favour of Blum's argument that these agents block the binding to the 6-amino group of the substrate.

If the dip in the curve for adenosine triphosphatase at 25° C. is due to the effect of the 6-amino binding site, then it is apparent that this site does not play a part at 0° C., since at this temperature the *p*H-activity curve of adenosine triphosphatase simulates a single dissociation curve. The similarity of the *p*H curves for adenosine and inosine triphosphates at 0° C., and the facts that *p*-chloromercuribenzoate inhibits adenosine triphosphatase at 0° C. and that adenosine triphosphate is hydrolysed more rapidly than inosine triphosphate at this temperature, all lend support to the view that the inhibitory 6-amino binding of adenosine triphosphate to the enzyme is somehow prevented at the low temperature.

It is possible to postulate a physical model which meets such a requirement if it is assumed that some configurational change is induced in the enzyme above a critical temperature, which must be higher than 0° C., and that this change is a necessary preliminary to the strong 6-amino binding. This change in configuration could be of the nature of a helix-coil transition, or a more specific effect induced by the binding of the substrate at its phosphate end. In the latter case, the effect of *p*-chloromercuribenzoate could be either to block the group which binds with the 6-amino of the substrate by combining with an –SH in its immediate vicinity, or to prevent the configurational change which must occur before the 6-amino group of the substrate and its combining site on the protein come into apposition. This concept of the enzymatic action of myosin on adenosine triphosphate may be expressed as follows :



(*M* represents the unchanged enzyme, *M**, the myosin of altered configuration, and *M** : ATP, the enzyme-substrate complex bound both at the P end and through the 6-amino position.)

The *p*H-activity curve of the enzyme which binds the substrate only at the triphosphate end, or which, at least, does not enter into the strong binding through the 6-amino position, still shows two distinct rising phases. This is seen in the *p*H curve for the hydrolysis of inosine triphosphate, or the hydrolysis of adenosine triphosphate by the enzyme treated with *p*-chloromercuribenzoate or 2,4-dinitrophenol. This curve may represent the activating effect of two dissociable groups on the enzyme, one with a *pK* of about 6·2, the other of about 9·0. At 0° C., two distinct phases are not apparent, but this effect could be the result of the *pK*'s of the two groups moving somewhat closer together. Some evidence that two different groups are still involved at 0° C. is seen in the difference between the adenosine triphosphatase curves at low (0·08 *M*) and high (0·5 *M*) potassium chloride concentrations. The flattening of the latter curve between *p*H's 8·0 and 9·5 suggests that the activating effect of the group with the higher *pK* has been eliminated at the higher potassium chloride concentration.

I am indebted to Dr. Manuel F. Morales, in whose laboratories this work was carried out, who made it possible by the award of a traineeship under the U.S. Public Health Service Training Grant programme (2*G*-174) and who suggested the study of *p*H dependence.

[1] Gilmour, D., and Griffiths, M., *Arch. Biochem. Biophys.*, **72**, 302 (1957).
[2] Szent-Györgyi, A., "Chemistry of Muscular Contraction", 2nd ed. (Academic Press, Inc., New York, 1951).
[3] Gellert, M., and Gilmour, D. (in preparation).
[4] Ouellet, L., Laidler, K. J., and Morales, M. F., *Arch. Biochem. Biophys.*, **39**, 37 (1952).
[5] Kielley, W. W., and Bradley, L. B., *J. Biol. Chem.*, **218**, 653 (1956).
[6] Morales, M. F., Osbahr, A. J., Martin, H. L., and Chambers, R. W., *Arch. Biochem. Biophys.*, **72**, 54 (1957).
[7] Morales, M. F., and Hotta, K. (in the press).
[8] Blum, J. J., *Arch. Biochem. Biophys.*, **55**, 486 (1955).
[9] Blum, J. J., and Felauer, E., *Arch. Biochem. Biophys.*, **81**, 285 (1959).
[10] Blum, J. J., *Fed. Proc.*, **18**, 13 (1959).
[11] Levy, H. M., Sharon, N., and Koshland, D. E., *Biochim. Biophys. Acta*, **33**, 288 (1959).

LETTERS TO THE EDITORS

PHYSICS

Thermodynamical Relations in Two-Component Two-Phase Systems

GIBBS[1], in his discussion of the phase rule, arrives at certain relations among the pressure, temperature and chemical potentials for two-component two-phase systems, making use of a fundamental equation now commonly known as the Gibbs–Duhem relation. A detailed account of this work has been given by Butler[2]. Since these systems have two degrees of freedom, the derivatives involving the intensive variables are of the form $(\partial x/\partial y)_z$. By introducing second-order Jacobians, the properties of the systems in question can be summarized compactly, and the results given by Gibbs and Butler readily obtained. The first systematic application of Jacobians to thermodynamical systems was made by Shaw[3]. The chief property required here is that :

$$(\partial x/\partial y)_z = J(x,z)/J(y,z)$$

where

$$J(x,z) = \begin{vmatrix} (\partial x/\partial \beta)_\gamma, & (\partial x/\partial \gamma)_\beta \\ (\partial z/\partial \beta)_\gamma, & (\partial z/\partial \gamma)_\beta \end{vmatrix}$$

β and γ being any two admissible variables.

The Gibbs–Duhem equations for a two-component two-phase system are :

$$S^{(i)}\mathrm{d}T - v^{(i)}\mathrm{d}p + m_1^{(i)}\mathrm{d}\mu_1 + m_2^{(i)}\mathrm{d}\mu_2 = 0 \quad (i = 1,2) \qquad (1)$$

where superscripts are used to distinguish the phases.

In Jacobian notation :

$$S^{(i)}J(T,x) - v^{(i)}J(p,x) + m_1^{(i)}J(\mu_1,x) + m_2^{(i)}J(\mu_2,x) = 0 \ (i = 1,2) \qquad (2)$$

On setting $x = p$, T, μ_1, μ_2, in turn, sets of homogeneous equations of the following form are obtained :

$$a_1y + b_1z + c_1w = 0$$
$$a_2y + b_2z + c_2w = 0$$

leading to the ratios :

$$\frac{y}{\begin{vmatrix} b_1 & c_1 \\ b_2 & c_2 \end{vmatrix}} = \frac{z}{\begin{vmatrix} c_1 & a_1 \\ c_2 & a_2 \end{vmatrix}} = \frac{w}{\begin{vmatrix} a_1 & b_1 \\ a_2 & b_2 \end{vmatrix}}$$

Writing

$$M = \begin{vmatrix} m_1^{(1)} & m_2^{(1)} \\ m_1^{(2)} & m_2^{(2)} \end{vmatrix} \qquad W = \begin{vmatrix} v^{(1)} & S^{(1)} \\ v^{(2)} & S^{(2)} \end{vmatrix}$$

$$S_1 = \begin{vmatrix} S^{(1)} & m_1^{(1)} \\ S^{(2)} & m_1^{(2)} \end{vmatrix} \qquad S_2 = \begin{vmatrix} S^{(1)} & m_2^{(1)} \\ S^{(2)} & m_2^{(2)} \end{vmatrix}$$

$$V_1 = \begin{vmatrix} v^{(1)} & m_1^{(1)} \\ v^{(2)} & m_1^{(2)} \end{vmatrix} \qquad V_2 = \begin{vmatrix} v^{(1)} & m_2^{(1)} \\ v^{(2)} & m_2^{(2)} \end{vmatrix}$$

and setting $J(p,T) = M\alpha$, we obtain the Jacobians given in Table 1. The Jacobian $J(x,y)$ is found at the intersection of the row labelled x and the column labelled y.

Applications. If $m_1^{(1)} = km_1^{(2)}$, and $m_2^{(1)} = km_2^{(2)}$, then $M = 0$, so that :

$$(\partial p/\partial \mu_1)_T = J(p,T)/J(\mu_1,T) = M/V_2 = 0$$
$$(\partial T/\partial \mu_1)_p = J(T,p)/J(\mu_1,p) = -M/S_2 = 0$$

Table 1. TABLE OF JACOBIANS

x ↓ y →	p	T	μ_1	μ_2
p	0	$M\alpha$	$-S_2\alpha$	$S_1\alpha$
T	$-M\alpha$	0	$-V_2\alpha$	$V_1\alpha$
μ_1	$S_2\alpha$	$V_2\alpha$	0	$-W\alpha$
μ_2	$-S_1\alpha$	$-V_1\alpha$	$W\alpha$	0

that is, if the composition of the phases is identical, then, at constant temperature, the pressure is in general a maximum or a minimum, and at constant pressure, the temperature is in general a maximum or a minimum (Gibbs–Konowalow laws).

Again, $M = 0$ implies $J(p,T) = 0$; that is, there is a functional relation between p and T, $p = \mathrm{f}(T)$. Hence, $\mathrm{d}p/\mathrm{d}T = (\partial p/\partial T)_{\mu_1} = S_2/V_2$, the same relation as for a one-component two-phase system (essentially the Clausius–Clapeyron equation).

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[1] Gibbs, J. W., "Collected Works", **1**, 99 (1928).
[2] Butler, J. A. V., "Commentary on the Scientific Writings of J. Willard Gibbs", **1**, 111 (1936).
[3] Shaw, A. N., *Phil. Trans. Roy. Soc.*, A, **234**, 299 (1935).

Cosmic Ray Intensity in Interplanetary Space

THE primary cosmic ray intensity at the Earth shows characteristic variations in time which are related to solar and geomagnetic activity. This relationship exists partly because of the modulation of the galactic cosmic ray flux by interplanetary magnetic and electric fields and partly because of the acceleration of particles to cosmic ray energies on or near the Sun. The eleven-year variation, the sharp drop in intensity associated with great magnetic storms, the quasi-periodic 27-day variation and the solar daily variation appear to belong to the former category. The occasional very large, and more frequent small increases in intensity, associated with solar flares are due to local production of high-energy particles. Detailed study of these variations in intensity has led to the development of several alternative models of the interplanetary magnetic field.

According to one of these models[1], the cosmic ray time variations can be explained in terms of a rather regular interplanetary magnetic field generated by current systems flowing in the solar corona and having a high degree of axial symmetry. This field would therefore be predominantly dipolar, and in order to explain the observed cosmic ray intensity variations it would need to have a strength of 10^{-5}–10^{-4} gauss near the Earth's orbit. The actual value would depend on the level of solar activity, the high value corresponding to sunspot maximum and the low value to sunspot minimum. It is further supposed in this model that the field contains small-scale

scattering centres so that low-energy galactic cosmic rays, which would otherwise be unable to reach the Earth, do so by a process of diffusion. The intensity of these particles at the Earth is reduced below the corresponding galactic intensity because at each collision with a magnetic inhomogeneity there is a finite probability that the particle will be scattered into such a direction that the field lines will guide it into the Sun, where it is absorbed. Thus the motion of the cosmic ray particles through the interplanetary field can be treated as a diffusion problem with a finite probability of absorption at each scattering collision.

Solution of the diffusion equation appropriate to this model leads to the following relation between φ_∞, the galactic cosmic ray flux, and φ_r, the flux at a point in the ecliptic plane distant r from the Sun:

$$\varphi_r = \varphi_\infty \left[\frac{pr^2}{45M_s} \right]^{\gamma(p)} \qquad (1)$$

M_s is the dipole moment of the current system, p is the particle rigidity in GV., and

$$\gamma(p) = \left[\left\{ 1 + 4K \left(\frac{p_0 + p}{p} \right)^2 \right\}^{\frac{1}{2}} - 1 \right] \Big/ 4$$

K and p_0 are constants which depend on the linear dimensions of the scattering irregularities and on their magnetic field strength. The values of M_s, K and p_0 all depend on the degree of solar activity, but their values can be deduced at any time from the cosmic ray intensity. Thus I have shown (ref. 1) that when the nucleonic intensity at Mount Washington, taken as a standard, is 25 per cent below its sunspot minimum value, the values of these constants are those given in column (*a*) of Table 1. This situation would correspond to a high level of activity near sunspot maximum. At an intermediate level of activity corresponding to a 13 per cent diminution of intensity at Mount Washington, the values of the parameters would be those given in column (*b*).

Table 1

Parameter	*a*	*b*
M_s (gauss cm.^{3})	3×10^{33}	2×10^{33}
K	0·30	0·25
p_0 (in GV.)	2·0	1·0

Using equation (1) and these values for the parameters, the cosmic ray intensity has been calculated as a function of distance from the Sun in the region between the orbits of Mars and Venus, and the result is shown in Fig. 1.

The variation in intensity to be expected over these distances depends very much upon the model of the interplanetary field we adopt. For example, the models proposed by Parker[2] and Beiser[3] would both lead to zero gradient in this region. On the other hand, if the cosmic rays are primarily produced in the Sun and trapped in the interplanetary field, we should presumably expect the intensity to increase with decreasing distance from the source, that is to say, the gradient would be opposite in sign to that calculated here.

It is to be expected that in the near future space probes will be launched towards both Mars and Venus (the space probe *Pioneer V* has now been launched towards Venus) and that these vehicles will carry cosmic ray detectors for the investigation of the spatial cosmic ray intensity dependence. These measurements should provide a crucial check on the validity of the interplanetary magnetic field models

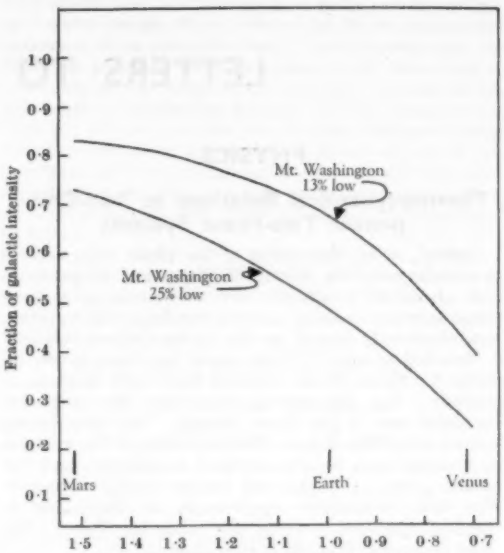

Fig. 1

developed from the study of cosmic ray intensity variations with time. Direct measurement of the magnetic fields by these probes is also likely, but cosmic ray intensity measurements provide information about the overall structure of the field, whereas magnetometer measurements relate only to the trajectory of the vehicle.

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[1] Elliot, H., *Phil. Mag.* (in the press).
[2] Parker, E. N., *Phys. Rev.*, **110**, 1445 (1958).
[3] Beiser, A., *J. Geophys. Res.*, **63**, 1 (1958).

Do Meteorites exist consisting predominantly of Feldspar?

In earlier communications[1] I have discussed the bluish fluorescence excited by ultra-violet light in many inorganic chemicals and minerals, a fluorescence which can be destroyed by strong annealing as well as by strong acids and which is most probably due to traces of organic substances; it can be regenerated by adding such traces to the annealed samples. As the fluorescence can also be observed in many magmatic minerals, one could think of applying this simple test for organic traces (or adsorbed OH-groups according to Ewles) also to meteorites.

The only earlier work known to me on the fluorescence of meteorites is that of Buddhue[2]. With ultra-violet excitation, his experiments were negative, but using cathode- and ion-rays he found bluish fluorescence in many meteorites; but as he states that, at least in one sample, the fluorescence survived "roasting for some time at low red heat in air", the carrier in this case can scarcely be an organic substance.

With ultra-violet excitation I, too, could observe no definite fluorescence in meteorites kindly lent me by the Natural History Museum and the Mineralogical Institute of the University of Vienna, and by the Max

Planck Institute of Chemistry (Otto Hahn Institute) in Mainz. This is not surprising, as these samples consist predominantly of olivine, and terrestrial olivines show no fluorescence either, probably because of their high iron content.

As many terrestrial feldspars show that fluorescence is of organic origin, it would be interesting to test meteorites consisting predominantly of feldspar (anorthite), if such exist. I should be grateful for any information as to whether such meteorites are known and whether I could have a small sample.

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[1] Przibram, K., *Nature*, **183**, 1048 (1959); *Naturwiss.*, **46**, 666 (1959); *Wien. Anz.* (Oct. 15, 1959 and March 3, 1960).
[2] Buddhue, G. D., *Amer. J. Sci.*, **239**, 839 (1941).

The Liquid Friction Constant

ONE of the more pressing problems in the kinetic theory of non-uniform liquids is the determination of the appropriate friction constant, β, in terms of the arguments of particle dynamics[1,2]. Although loosely termed a friction constant, β is more correctly a tensor quantity: it is likely (on clear physical grounds) to have its largest value in the region of melting and to decrease steadily towards the condensation region, although how strongly is not fully clear[2]. Any accurate calculation of β, made without serious approximation, offers extreme mathematical difficulties that have not yet been overcome. A rough theoretical estimate of the order of magnitude of β may, however, be possible in the melting region without a direct appeal to the full mathematical details of the theory of non-equilibria, as will now be shown. The method involves a relation proposed by Prof. E. N. da C. Andrade in *Nature*[3] some time ago.

According to Andrade, the viscosity, η, thermal conductivity, k, and specific heat at constant volume, C_v, for a non-metallic monatomic liquid at the melting point, are related according to the expression:

$$k = A\eta C_v \qquad (1)$$

Here A, a constant, is approximately unity: for liquid argon, Andrade finds A to be about 0·8. In what follows I shall accept this relation as being true.

Current ideas of liquid non-equilibria describe the irreversible movement to equilibrium in terms of β alone[4]: in particular, liquid viscosity and thermal conductivity are derived in the forms:

$$\eta = \beta X_1 \text{ and } \beta k = X_2,$$

where X_1 and X_2 are functions derivable from a knowledge of the liquid equilibrium structure. It follows then that the ratio η/k is given by:

$$\frac{\eta}{k} = \beta^2 \frac{X_1}{X_2} \qquad (2)$$

Alternatively, C_v is known to be given by $C_v = \left.\frac{\partial U}{\partial T}\right|_v$, where U is the internal energy, related by the well-established principles of statistical mechanics to the free energy, F, and the partition function Z according to:

$$F = -kT \log(\lambda Z); \quad h^{3N}\lambda = (2\pi mkT)^{3N/2} \qquad (3)$$

the symbols in λ having their usual meaning.

Combining (1), (2) and (3), there results an expression for β:

$$\beta \sim \left(-T\frac{X_1}{X_2}\frac{\partial^2 F}{\partial T^2}\right)^{-1/2} \qquad (4)$$

where $\sqrt{A}$ has been assumed equal to unity. If X_1, X_2 and F are known numerically, (4) can be expected to give an estimate of the order of magnitude of β in the region where its value is largest, that is, in the neighbourhood of the melting point. A comparison with the independent value of β in the vapour region will then give some information about the mean dependence of β on the temperature.

In the absence of any full theory of melting, it is probably sufficient to assume that the liquid short-range order is adequately represented by the pair distribution, $n^{(2)}$, even in the neighbourhood of the melting temperature. If this is accepted, the functions X_1 and X_2 may be calculated[5,6] if $n^{(2)}$ is known[7]. On the other hand, with $n^{(2)}$ known, I have recently shown how the liquid free-energy for some temperature T can be determined by the mathematical procedure of variable interparticle coupling[8]. Consequently, if $n^{(2)}$ is known, β can be determined from (4) directly without appeal to experimental data. The reliability of the value of β so derived will depend upon the adequacy of $n^{(2)}$ as a representation of the liquid structure in the melting region; however, (4) will still apply even if distributions of higher order are also important.

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[1] Eisenschitz, R., *Nature*, **167**, 216 (1951); *Proc. Roy. Soc.*, A, **216**, 29 (1952).
[2] Turner, R. E., *J. Chem. Phys.*, **29**, 856 (1958).
[3] Andrade, E. N. da C., *Nature*, **170**, 794 (1952).
[4] Eisenschitz, R., "Statistical Theory of Irreversible Processes" (Oxford Library of Physical Sciences, 1958).
[5] Eisenschitz, R., *Proc. Phys. Soc.*, A, **190**, 455 (1949).
[6] Eisenschitz, R., and Boot, A., *Proc. Phys. Soc.*, **74**, 208 (1959).
[7] Cole, G. H. A., *Proc. Phys. Soc.*, **73**, 713 (1959).
[8] Cole, G. H. A., *Proc. Phys. Soc.* (in the press).

Orientation of Stearic Acid Monolayers on Silver Single Crystals

ELECTRON diffraction has been used to study stearic acid monolayers adsorbed by retraction from *n*-hexadecane solution on a flat (111) face of a silver single crystal. The crystal was prepared by evaporating silver on to a heated mica substrate[1].

Fig. 1 shows the diffraction pattern of the silver substrate in the $1\bar{1}0$ azimuth. The elongation of diffraction spots into streaks normal to the edge of the shadow shows the surface to be very flat. This should be compared with Fig. 2, which shows the diffraction pattern when a stearic acid monolayer has been retracted on the silver, with the crystal in the same azimuth. The silver pattern has been reduced in intensity and additional streaks appear half-way between the substrate pattern. Fig. 3 shows a diffraction pattern with the silver in the $2\bar{1}\bar{1}$ azimuth with further extra streaks due to the monolayer.

These patterns are different from any previously observed from monolayers adsorbed on polycrystalline substrates, which have certainly been rough on a molecular scale and have shown only layer lines indicating orientation of the hydrocarbon chains approximately perpendicular to the substrate. They

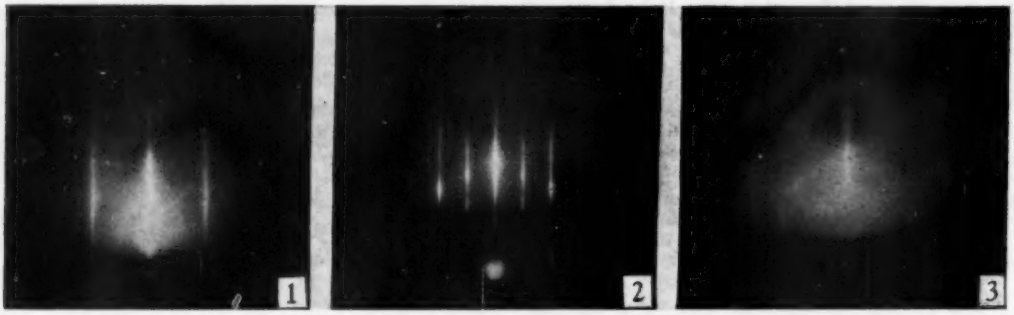

Fig. 1. Diffraction pattern of a (111) face of silver single crystal in $1\bar{1}0$ azimuth. The elongation of spots into streaks normal to the edge of the shadow indicates that the surface is extremely smooth

Fig. 2. Diffraction pattern from a stearic acid monolayer adsorbed on a (111) face of silver single crystal in $1\bar{1}0$ azimuth. Extra streaks appear halfway between the substrate pattern, showing the monolayer to be oriented on the silver lattice

Fig. 3. The same as Fig. 2, but with the silver in the $2\bar{1}\bar{1}$ azimuth. Extra streaks due to the monolayer are apparent

are, however, similar to those found by Hagihara *et al.*[2] when alkyl xanthates and dithiophosphates are adsorbed on a cleavage face of galena; these were attributed to adsorption on lead atoms in the galena lattice. Figs. 2 and 3 show that the monolayer has a regular lateral arrangement of molecules oriented on the silver substrate in the manner shown in Fig. 4. The individual monolayer patches may take up one of three orientations consistent with the three-fold symmetry of the (111) face of silver. The hydrocarbon chains are oriented normal to the surface.

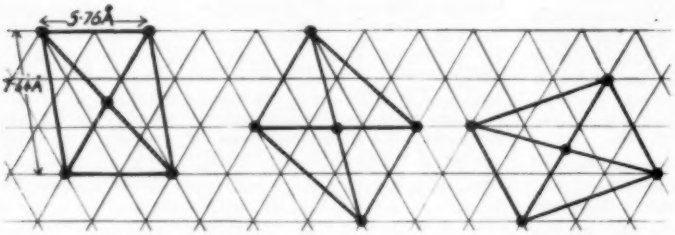

Fig. 4. Orientation of the stearic acid on the silver lattice. Three possible orientations of the individual monolayer patches are illustrated

This arrangement of stearic acid molecules is different from either the α- or β-modifications of the bulk crystals, where the basal plane is rectangular, but it does give an area per molecule of 21·6 Å.^{2}; this is reasonably close to 21·3 Å.^{2}, which would be the value for a monolayer with the β-configuration of stearic acid.

The intensities of the diffraction streaks in Fig. 2 suggest that the silver is contributing to the pattern. This is consistent with the previous observation[3] that only one-third of the surface is covered by stearic acid molecules, due to incorporation of *n*-hexadecane in the monolayer.

I am grateful to Dr. D. W. Pashley for preparing the silver crystals, to Drs. F. P. Bowden and D. Tabor for continued encouragement, and to Messrs. Joseph Lucas for a research grant.

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[1] Pashley, D. W., *Phil. Mag.*, **4**, 216 (1959).
[2] Hagihara, M., and Uchikoshi, H., *Nature*, **174**, 80 (1954).
[3] Mathieson, R. T., *Nature*, **183**, 1803 (1959).

A New Predissociation in Nitrogen

In a previous paper a high-resolution study of the fifth positive system of nitrogen was presented[1]. It was there mentioned that no bands with v' greater than 2 have been observed, although some of them, according to the Franck–Condon principle, should be strong enough to be seen. This fact was suggested as being due to a possible predissociation, but since the exact location of the states involved in this transition, $x^1\Sigma_g^+ - a'^1\Sigma_u^-$, was not known, nothing more definite could be said about the nature of such a predissociation. Recently, however, Wilkinson and Mulliken[2] obtained accurate term values for these states, and I found it therefore of interest to reinvestigate my plates in order to study the possible predissociation in more detail.

Combining the new term value $T_{00}(x^1\Sigma_g^+) = 113{,}211{\cdot}0$ cm.$^{-1}$ with the vibrational and rotational constants given before ($\Delta G_{1,2} = 1{,}868{\cdot}4$, $\Delta G_{2,3} = 1{,}827{\cdot}2$, and $B_2 = 1{\cdot}694$ cm.$^{-1}$), it is now found that the $v' = 3$ level is at 118,693 cm.$^{-1}$; that is, just above the dissociation limit $^2D + {}^2D$ at 117,170 cm.$^{-1}$ (assuming 9·756 eV. for the dissociation energy). On the other hand, the $v' = 2$ level is at 116,907 cm.$^{-1}$; that is, just below the same limit. The rotational lines lying closest to the dissociation limit should then correspond to about $J' = 13$–15. Consequently, if the breaking-off of the vibrational structure is due to a predissociation by a state resulting from ^2D atoms, a breaking-off of the rotational structure in the $v' = 2$ level, above the J values just mentioned, should be expected as well.

Most of the bands belonging to the $v' = 2$ progression are much obscured by stronger structure, but two of them, the (2·3) and (2·9) bands, were sufficiently resolved to permit an extension of the branches beyond the point reported before. The wave numbers of the lines of these two bands, together with combination differences for the upper level, are given in Table 1. The intensity distribution in the two bands is shown in Fig. 1.

In all four branches there was a breaking-off above $J' = 25$ (118,005 cm.$^{-1}$), or at least the lines were too

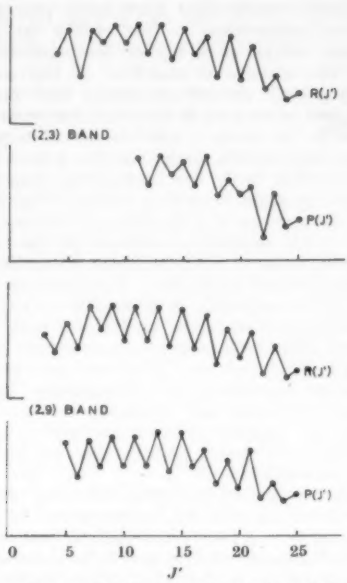

Fig. 1. Intensity distribution in the (2·3) and (2·9) bands of the Fifth Positive System. (The curves are constructed directly from photometer tracings)

weak to be identified. However, the breaking-off cannot be quite sharp, because there is a clear intensity drop in many of the preceding lines, as seen in Fig. 1. It is not possible to tell with certainty where the intensity anomaly begins, but it is not unlikely that it is around $J' = 13 - 15$; that is, just close to the dissociation limit as indicated above.

From the selection rules for predissociation it is to be expected that only $^{1,3}\Sigma_g^-$ or $^{1,3}\Pi_g$ states can perturb a $^1\Sigma_g^-$ state. According to Mulliken[3], the only states of these types resulting from two ^2D atoms are two $^3\Sigma_g^-$ and two $^3\Pi_g$, all repulsive or loose. It may therefore be suggested that the observed breaking-off of the vibrational and rotational structure in the $x^1\Sigma_g^-$ state above $v = 2$, $J =$ about 15, is due to a predissociation by a $^3\Sigma_g^-$ and/or a $^3\Pi_g$ state dissociating into $^2D + {}^2D$ atoms with dissociation limit at 117,170 cm.$^{-1}$, forbidden only by the less rigorous spin selection rule.

Table 1. Wave Numbers and Combination Differences for Bands of the Fifth Positive System of Nitrogen

	Band (2·9)			Band (2·3)		
J	$P(J)$	$R(J)$	Δ_2F'	$P(J)$	$R(J)$	Δ_2F'
0	—	36,453·13*		—	*	
1	36,446·73*	457·21*	10·48	*	44,728·18	
2	444·99*	462·12	17·13	*	*	
3	443·92*	467·72*	23·80	*	737·87	
4	443·56*	474·07	30·51	*	743·45*	
5	443·92*	481·13	37·21	*	749·58	
6	444·92*	488·92	44·00	*	756·19	
7	446·73	497·55	50·82	*	*	
8	449·26	506·84	57·58	44,713·50*	771·09*	57·59
9	452·53	516·86	64·33	715·08*	779·39	64·31
10	456·53	527·65	71·12	717·14*	788·22	71·08
11	461·27	539·13	77·86	719·74	797·59	77·85
12	466·76	551·39	84·63	722·88*	807·46	84·58
13	472·99	564·39	91·40	726·50	817·89	91·39
14	479·96*	578·12	98·16	*	828·92	
15	487·67	592·56	104·89	735·62*	840·44	104·82
16	496·14	607·76	111·62	740·87	852·46	111·59
17	505·36	623·66	118·30	746·71	865·06	118·35
18	515·22	640·33	125·11	753·14*	878·21	125·07
19	525·90	657·71	131·81	760·07	891·83	131·76
20	537·30	675·89	138·59	767·63	906·00	138·37
21	549·51	694·61*	145·10	775·64	920·72*	145·08
22	562·42	714·39	151·97	784·39*	935·99	151·60
23	576·00	734·86*	158·86	793·25	951·66*	158·41
24	590·38	755·68*	165·30	802·97	968·50*	165·53
25	605·45			813·05		
26	621·28			825·10*		

* Indicates blended lines.

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[1] Lofthus, A., *J. Chem. Phys.*, **25**, 494 (1956).
[2] Wilkinson, P. G., and Mulliken, R. S., *J. Chem. Phys.*, **31**, 674 (1959).
[3] Mulliken, R. S., in "The Threshold of Space", 173 (Pergamon Press, Inc., New York, 1957).

METALLURGY

A Trace Element Effect in Alloys based on the Aluminium–Zinc–Magnesium System

The remarkable changes in properties which may be achieved by the addition of small quantities, or traces, of certain elements to age-hardening alloy systems has aroused much interest recently. A notable example is the pronounced effect of traces of cadmium, indium, or tin on the elevated temperature ageing characteristics of aluminium–copper alloys[1]. Similar observations have been made in a number of alloys; but, with the exception of a study of trace element additions to ternary aluminium–copper–lithium alloys[2], investigations have been confined to binary systems.

We have been studying ageing phenomena in alloys based on the aluminium–zinc–magnesium system and have found what appears to be a similar effect in these complex materials. The early stages in this project were concerned with observations of the ageing characteristics of a wide range of the high-purity, ternary alloys[3]. This work has now been extended to include a study of the influence of quaternary and quinary additions to several of these ternary alloys. A very interesting result is that small amounts (up to 0·1 at. per cent) of silver may exert a marked effect both on the properties obtained by age-hardening and on the structural changes associated with the processes. These effects were first detected on adding silver to certain of the ternary alloys. It was found afterwards that the effects were still apparent if silver was added to certain high-purity aluminium–zinc–magnesium–copper alloys, and to the more complex commercial material conforming to the specification *D.T.D.* 683 (aluminium: 5·8 per

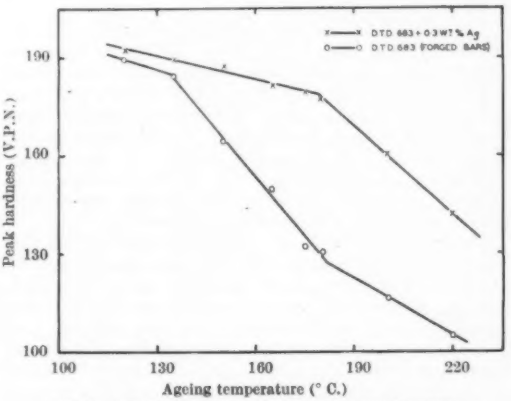

Fig. 1. Peak hardness/ageing temperature relationships

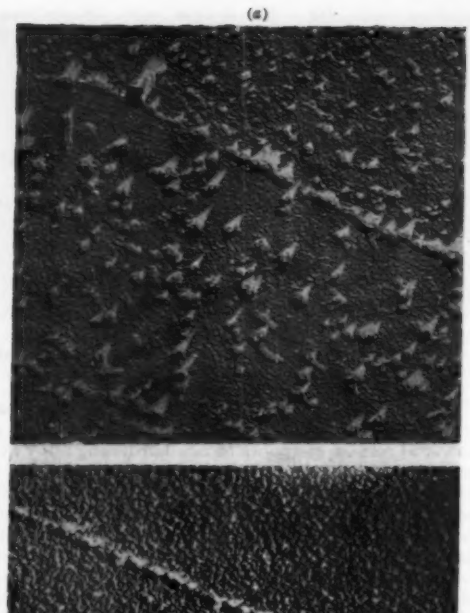

Fig. 2. The effect of silver on the microstructure of an alloy aluminium–4 per cent zinc–3 per cent magnesium–1 per cent copper. Alloys aged 1 day at 175° C. Electron micrographs (× 25,000). *a*, No silver. Hardness 108 *V.P.N.* Approx. particle size 300–900 Å. *b*, 0·3 weight per cent silver. Hardness 150 *V.P.N.* Approx. particle size less than 300 Å.

cent zinc–2·4 per cent magnesium–1·4 per cent copper–0·31 per cent manganese–0·12 per cent chromium–0·25 per cent iron–0·17 per cent silicon–0·05 per cent titanium).

An indication of the property changes can be obtained from Fig. 1, in which the peak hardness values from hardness-time curves for the alloys *D.T.D.* 683 and *D.T.D.* 683 + 0·3 weight per cent silver are plotted against the ageing temperature. It will be seen that, in alloy *D.T.D.* 683, the capacity for age-hardening decreases rapidly as the ageing temperature is raised above 135° C. With the addition of silver, however, the high level of peak hardness is maintained up to a temperature of 180° C.

The effect of silver on the microstructure of this family of alloys can be seen by comparing Figs. 2*a* and 2*b*. In this case silver has been added to a high purity, quaternary alloy, aluminium–4 per cent zinc–3 per cent magnesium–1 per cent copper, and the alloys aged at 175° C. Greater hardening was again recorded in the alloy containing silver, and this seems clearly to have its origin in the much finer dispersion of precipitate in this material.

The above results may have both practical and theoretical significance. The ability to age the commercial alloys at a higher temperature should increase the structural stability of the materials. This may benefit several properties such as fatigue strength and behaviour at elevated temperatures. A reduction in the level of residual stress in quenched and aged components might also be expected. This factor, together with the more even dispersion of precipitate in grain boundary regions (Fig. 2*b*), may increase the resistance of the alloys to stress-corrosion cracking. Of theoretical interest is the fact that silver must, in some way, facilitate nucleation of the precipitate formed by ageing. Furthermore, in these more complex materials, it is possible by varying the composition to observe the effects of a trace element on several different precipitation processes occurring in the same alloy system. This may provide further information concerning the mechanism by which these trace elements have their effects.

Both these aspects of the investigation are being studied in more detail and further results will be reported shortly.

Thanks are due to the Chief Scientist, Australian Defence Scientific Service, Department of Supply, for permission to publish this communication and to Mr. K. R. Sargant, who prepared the electron micrographs.

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[1] Hardy, H. K., *J. Inst. Metals*, **78**, 169, 657 (1950–51); **80**, 483 (1951–52).
[2] Hardy, H. K., *J. Inst. Metals*, **84**, 429 (1955–56).
[3] Polmear, I. J., *J. Inst. Metals*, **86**, 113 (1957–58).

Allotropic Transformations in Plutonium

Information on the allotropy of plutonium is available from several laboratories[1,2]. There is general agreement about the behaviour of specimens undergoing changes of heat content at the rates usually employed, and there is general and tacit agreement to ignore the large hysteresis between heating and cooling effects, using the heating temperatures only in alloy equilibrium diagram work. That equilibrium is not available, even in pure plutonium, at these temperatures is clear from the published work on transformation kinetics[3,4].

Rate of transformation was read from a record of dilatation against time, using a transducer system capable of high sensitivity over a wide range and of proved stability. The temperature, which was maintained constant for long enough to establish a definite rate, was recorded on the same chart as the e.m.f. produced by a chromel-alumel thermocouple, and this was also measured at frequent intervals on a portable potentiometer. The sensing head of the thermocouple was cemented in a 0·025-in. diameter hole drilled on a diameter of a cylindrical specimen about 0·3 in. in diameter and about 0·65 in. long, about 0·15 in. from one end. The glass-fibre insulated leads were wound four times round the specimen before being led past the silica push rod to the cold junction, which was maintained at a constant temperature by a mercury-and-toluene-in-glass regulator. After the full transformation size had been determined, the specimen was

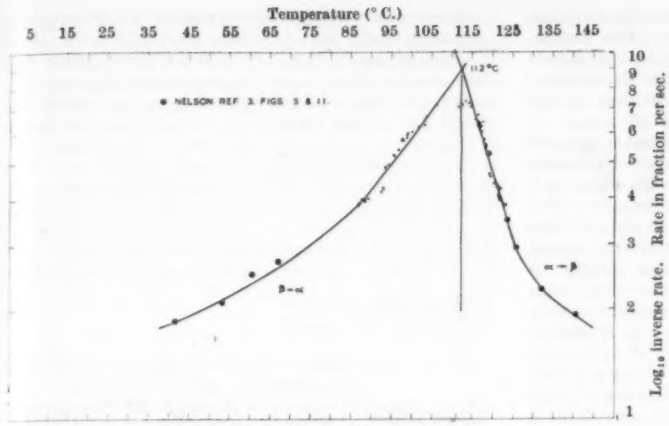
Temperature (° C.)
5 15 25 35 45 55 65 75 85 95 105 115 125 135 145
112°C
● NELSON REF. 3. FIGS. 5 & 11.
β→α
α→β
10 9 8 7 6 5 4 3 2 1
Log10 inverse rate. Rate in fraction per sec.

Fig. 1

kept at about the 1 : 1 phase composition and the temperature remained within the range under study.

Log_{10} of the inverse of the rate of transformation was plotted on a logarithmic scale against temperature; the unit chosen for the rate, fraction of the transformation per second, caused this log–log–inverse plot to fall conveniently into one decade. The more prominent points on the graphs were computed from curves published by Nelson[3,4] and supplement this work. The two branches in Fig. 1 indicate a temperature of 112° C. ± 1° C. as the temperature of thermodynamic equilibrium for the $\alpha \rightleftharpoons \beta$ transformation; the points shown above 7 are at rates comparable with background effects in our equipment. In Fig. 2 there is rather more scatter, but a temperature of 184° C. ± 2° C. is predicted as the equilibrium temperature for the $\beta \rightleftharpoons \gamma$ transformation. The corresponding transformation-rates are 10^{-2} and about 10^{-5} less than the lowest rates measurable on our equipment, are about 10^{-5} to 10^{-8} lower than the lowest rates available to Nelson, and appear to be 10^{-6} to 10^{-9} below the rates forced on plutonium by the techniques usually employed.

The sluggishness of the $\beta \rightarrow \alpha$ transformation, mentioned for example by Abramson[1], is reflected in the low slope of the log–log–inverse rate plot. The slope of the corresponding plot for $\delta \rightarrow \gamma$ is possibly even lower, and as the $\gamma \rightarrow \delta$ transformation follows the pattern set by the $\alpha \rightarrow \beta$ and $\beta \rightarrow \gamma$, the temperature for the $\gamma \rightarrow \delta$ equilibrium is probably quite close to 310° C.

To judge from the departure from the equilibrium temperature required to produce a given rate of transformation at these relatively low rates of transformation, the β-phase is more easily formed from either of the other two phases than they are formed from β-phase.

The thermocouple was checked against ice to establish the cold junction correction, and the wire was checked against gallium, indium and tin. Calibration error is not likely to exceed 1 deg. C.

I wish to thank Drs. J. A. Lee and M. B. Waldron for their interest, and Mr. D. E. Dulieu for his assistance.

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[1] Second United Nations International Conference on the Peaceful Uses of Atomic Energy, Abramson *et al.*, paper *P*/327; Konobeevsky, S. T., *et al.*, paper P/2230; Waldron, M. B., *et al.*, paper *P*/71.

[2] Coffinberry, A. S., *et al.*, "Progress in Nuclear Energy", 1, Ser. V, Chap. 4 (Pergamon Press, 1956).

[3] Nelson, R. D., "Transformation Kinetics of Plutonium", Part 1, "A Study of the $\beta \rightarrow \alpha$ and $\alpha \rightarrow \beta$ Transformations", U.S. Atom. Energy Comm., Report *HW* 55778.

[4] Nelson, R. D., "Transformation Kinetics of Plutonium", Part 2, "A Study of the $\gamma \rightarrow \beta \rightarrow \alpha$ and $\alpha \rightarrow \beta \rightarrow \gamma$ Transformations", U.S. Atomic Energy Comm., Report *HW* 56843 (1958).

CHEMISTRY

Determination of Adsorption in Double Layers involving Solid Metals

Investigations of effects of adsorption on the mechanism of electrode processes, as encountered in corrosion inhibition, electrodeposition, etc., require determinations of the amounts adsorbed on an electrode *in situ* simultaneously with electrochemical measurements on the system. Previous work in this field has been ineffective because: (1) the adsorption has been determined on specimens different from those used in the electrochemical measurements; and (2) the amounts adsorbed have been determined subsequent to the removal of the specimen from the solution and washing of the surface, thus restricting the adsorbed amounts to those remaining under conditions undefined with respect to potential and the effect of washing.

A method developed in this Laboratory enables determination of the adsorption at solid metal – solu-

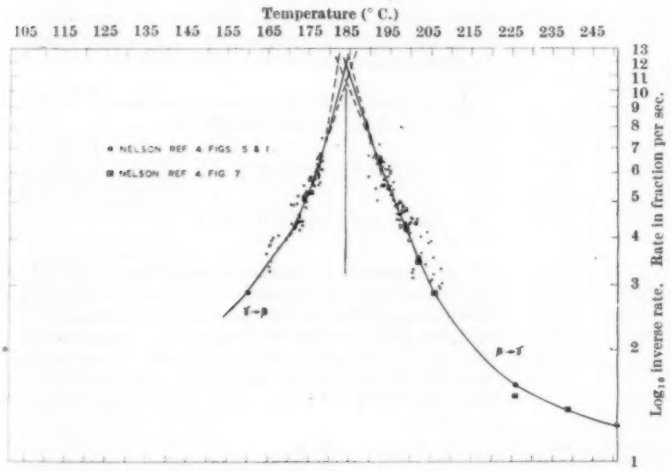
Temperature (° C.)
105 115 125 135 145 155 165 175 185 195 205 215 225 235 245
● NELSON REF. 4. FIGS. 5 & 7.
⊕ NELSON REF. 4. FIG. 7.
γ→β
β→γ
13 12 11 10 9 8 7 6 5 4 3 2 1
Log10 inverse rate. Rate in fraction per sec.

Fig. 2

tion interfaces simultaneously with electrochemical measurements on the same specimen. The importance of such work has been illustrated by Schwabe[1]. For example, the method makes possible the evaluation of the quantity adsorbed as a function of the metal – solution potential difference.

The adsorption is determined by a method suggested in principle by Aniansson[2]. A thin layer of a metal is deposited on the external side of a mica foil which forms the window of a gas proportional counter. The counter is mounted above the surface of the solution containing the labelled absorbate so that the metal film and the surface of the solution are parallel to one another. The distance between the metal film and the solution is decreased in stages, and the count-rate is determined as a function of the distance. When the solution comes into contact with the metal film, the count-rate increases by an amount corresponding to the formation of the adsorbed layer. The value of the activity of the amount adsorbed is obtained from the difference between the count-rate after the contact has been established and the count-rate before the contact is made, extrapolated to zero distance. The amount adsorbed is found by measuring the activity of a known mass under geometrical conditions limitingly close to those of the adsorption experiment.

The electrochemical parameters of the metal – solution interface are determined by steady-state potential measurements, using a Haber–Luggin capillary which ends near the surface of the metal film. Alternatively, resistance, activation and concentration over-potentials in the presence of the adsorbate are determined by d.c. transient techniques[3]. During a single pulse of c.d. $\leqslant$30 m.amp./cm.^{2} and a duration $\leqslant$1 m.sec., only 0·1 of a monolayer of the metal surface is dissolved or deposited, so that the surface coverage of the adsorbate is not essentially disturbed by the pulse, and refers to the condition before its application.

Fig. 1 shows the results of a typical adsorption experiment. From A to B the distance between the

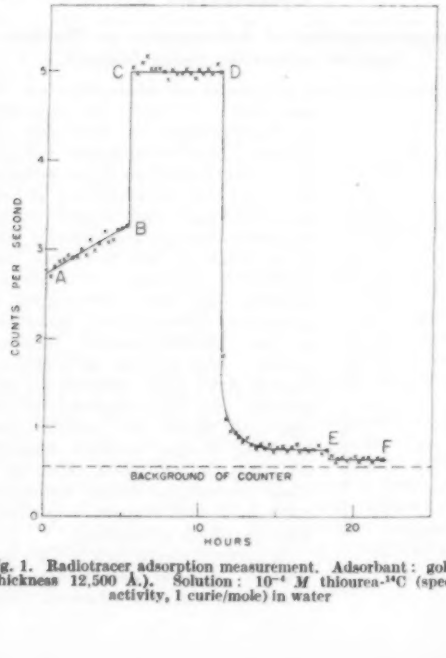

Fig. 1. Radiotracer adsorption measurement. Adsorbant: gold (thickness 12,500 Å.). Solution: 10^{-4} M thiourea-^{14}C (spec. activity, 1 curie/mole) in water

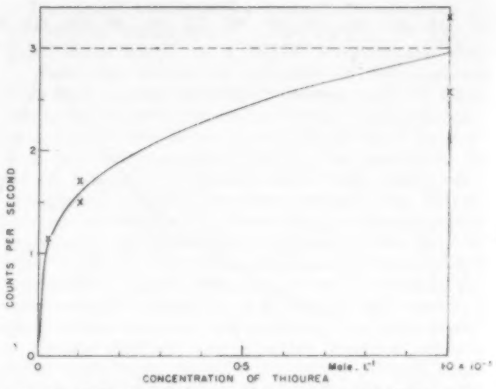

Fig. 2. Adsorption isotherm for thiourea on gold from aqueous solution at 28° C. A monolayer corresponds to *c.* 3 counts per sec.

metal film and the surface of the solution was decreased gradually. At B the solution came into contact with the film, resulting in an increase of the count-rate to C. At D the radioactive solution was replaced by distilled water, causing a decrease of the count-rate. The water was renewed at E. The abrupt change in count-rate from B to C shows that the adsorption is complete in a fraction of the counting time used (1,000 sec.). Since F is close to the inherent background of the counter, it is concluded that the adsorption is highly reversible.

Fig. 2 gives a preliminary adsorption isotherm for thiourea from aqueous solution. The reproducibility between different experiments is 0·1 of a monolayer at concentrations $\leqslant 2 \times 10^{-4}$ mole litre^{-1}, while the method encounters greater difficulties at higher concentrations. With improvements which are at present under way, a precision of 0·1 of a monolayer, or *c.* 3×10^{-11} mole cm.$^{-2}$, is expected at concentrations up to *c.* 2×10^{-3} mole litre^{-1}.

Results of the corresponding electrochemical measurements will be given elsewhere.

The number of disintegrations per second originating from an adsorbed layer is given by the formula:

$$N_{\text{ads.}} = \tfrac{1}{2} \times 3{\cdot}7 \times 10^{10} \times A \times \theta \times \Gamma_m \times \alpha \quad (1)$$

where A is the effective surface area (in sq. cm.), θ the degree of coverage, Γ_m the amount of adsorbate (in mole. cm.$^{-2}$) corresponding to 1 monolayer, and α the specific activity of the adsorbate (in curies per mole).

The number of disintegrations per second from the solution is given approximately by:

$$N_{\text{sol.}} = \tfrac{1}{2} \times 3{\cdot}7 \times 10^{10} \times A \times c \times \alpha \times \int_0^\infty \exp(-\mu x)\mathrm{d}x$$

$$= \tfrac{1}{2} \times 3{\cdot}7 \times 10^{10} \times A \times c \times \alpha \times \frac{1}{\mu} \quad (2)$$

(cf. ref. 4), where c is the concentration of the adsorbate in the solution (in mole cm.$^{-3}$), and μ is the linear absorption coefficient for the emitted radiation in the solution (for carbon-14 β-particles, approximately 320 cm.$^{-1}$ in water[5]). From (1) and (2) one derives for a 10^{-3} M solution in water of a compound labelled with carbon-14 with $\Gamma_m = 3 \times 10^{-10}$ mole/cm.^{2}, and $\theta = 0{\cdot}1$:

$$\frac{N_{\text{ads.}}}{N_{\text{sol.}}} \simeq 1 \text{ per cent}$$

a result which indicates the necessity for accuracy of the order of 0·1 per cent in the recorded count-rate, with stability to this amount over several hours.

Application of values of μ to equation 2 shows that the method is applicable to substances labelled with carbon-14, sulphur-35, calcium-45 and iodine-131.

A description of the instrumentation developed to meet the requirements of stability and precision of the counter and the associated electronic equipment, as well as a full report of the work, will be published elsewhere.

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[1] Schwabe, K., *Chem. Tech.* (Berlin), **10**, 469 (1958).
[2] Aniansson, G., *J. Phys. Chem.*, **55**, 1286 (1951). Cf. also, Cook, H. D., *Rev. Sci. Instr.*, **27**, 1081 (1956), and Cook, H. D., and Ries, jun., H. E., *J. Phys. Chem.*, **63**, 226 (1959).
[3] Mattsson, E., and Bockris, J. O'M., *Trans. Farad. Soc.*, **55**, 1586 (1959).
[4] Cf. Libby, W. F., *Anal. Chem.*, **29**, 1566 (1957). Hendler, R. W., *Science*, **130**, 772 (1959).
[5] Karnovsky, M. L., Foster, J. M., Gidez, L. I., Hagerman, D. D., Robinson, C. V., Solomon, A. K., and Villee, C. A., *Anal. Chem.*, **27**, 852 (1955).

Infra-red Spectra of Deuterium-labelled Carbohydrates

IN a previous report[1] we showed that the stretching frequency of the axial C_1—D in the 1-deutero derivatives of β-D-glucose and β-D-mannose was about 40 cm.$^{-1}$ lower than the corresponding equatorial C_1—D in the 1-deutero derivatives of α-D-glucose and α-D-mannose. Examination of a further series of 1-deutero carbohydrate derivatives (see Table 1) has revealed that this is a general feature of hexopyranose derivatives and that the stretching frequencies of the equatorial C_1—D groups of two crystalline D-pentopyranoses also fall in the same range as those of other equatorial groups. Although these stretching frequencies (some of which appear double) were measured with a sodium chloride prism and not therefore with great accuracy, the consistent differences were beyond experimental error and it was possible to resolve the frequencies of both anomers in the spectra of impure samples. The C—H stretching frequencies were not resolved by the infra-red spectrometer used in this work; the frequency of the approximate centre of the C—H stretching band was used in calculating the ratios recorded in Table 1.

The above results are similar to those observed for the C—D stretching frequencies in *cyclo*hexane[2] and in steroids[3]. Other stretching vibrations (for example, C—O—C stretching of 3-O-acetoxy groups in steroids[4], C—O stretching of 3-hydroxy groups in steroids[5] and the C—O—S stretching of O-sulphate groups in sugar sulphates[6] and the chondroitin sulphates[7,8]) appear to occur at different frequencies for equatorial and axial substituents attached to six-membered rings. In 3-hydroxy steroids[5] this phenomenon has been explained by suggesting that in the equatorial vibrations the motion of the C_3 atom along the C—O axis involves an appreciable expansion and contraction of the *A* ring, whereas for the axial substituent the motion is largely normal to the plane of the ring. Under these conditions the restoring force acting on the C_3 atom might be less for the axial than for the equatorial motion and hence induce the lower vibration frequency. However, other factors may be involved (cf. C—H stretching frequencies of perfluoro*cyclo*hexane derivatives[9]).

From a comparison of the spectra of normal and C_1-deutero sugars it was found that the C_1—H deformation vibrations were reasonably characteristic[1] in the free sugars and could be distinguished from other C—H deformation vibrations in the molecule. In both anomers of all the hexoses (see Table 1) the higher C_1—H deformation frequencies occurred in the narrow range 1,363–1,387 cm.$^{-1}$, but there was no consistent difference between the anomers. The lower C_1—H deformation frequencies covered a much wider range. While the range of the α-anomers (1,275–1,315 cm.$^{-1}$) only just overlapped that of the β-anomers (1,312–1,340 cm.$^{-1}$), this vibration was not sufficiently characteristic or conspicuous to be of use for diagnosis.

We are indebted to Prof. E. J. Bourne and Dr. H. Weigel for collaboration in the early part of this

Table 1. STRETCHING AND BENDING FREQUENCIES (CM.$^{-1}$) OF $C_{(1)}$—H AND $C_{(1)}$—D

Pyranose sugar	Stretching frequencies			Deformation frequencies					
				Higher			Lower		
	$C_{(1)}$—H	$C_{(1)}$—D	Ratio	$C_{(1)}$—H	$C_{(1)}$—D	Ratio	$C_{(1)}$—H	$C_{(1)}$—D	Ratio
α-D-Glucose*	*c.* 2,910*s*	2,180*m*	*c.* 1·34	1,375*s*	1,095*s*	1·26	1,284*m*	965*s*	1·33
β-D-Glucose†	*c.* 2,920*s*	2,140*m*	*c.* 1·36	1,363*s*	1,072*s*	1·27	1,312*s*	972*s* 965*s*	1·35 1·36
α-D-Glucose pentaacetate*	*c.* 2,950*s*	2,245*m*	*c.* 1·31	1,382*s*	1,102*s*	1·26	1,333*w*	964*s*	1·38
β-D-Glucose pentaacetate†	*c.* 2,950*s*	2,120*m*	*c.* 1·39	1,390*s*	1,052*m*	1·32	1,333*m*	993*m*	1·34
Methyl α-D-glucoside*	*c.* 2,950*m*	2,200*w*	*c.* 1·34	1,366*w*	998*m*	1·37	1,325*w*	983*m*	1·35
Methyl β-D-glucoside†	*c.* 2,950	2,095 2,140	*c.* 1·39						
Methyl β-D-glucoside tetra-acetate†	*c.* 2,980*s*	2,120*w*	*c.* 1·41	1,415*w*	1,068*m*	1·33	1,382*s*	1,038*s*	1·33
α-D-Mannose*	*c.* 2,900*s*	2,200*m*	*c.* 1·32	1,372*s*	1,000*s*	1·37	1,275*s*	912*m*	1·40
β-D-Mannose†	*c.* 2,860*s*	2,155*m*	*c.* 1·33	1,367*m*	1,106*s*	1·24	1,324*s*	969*s*	1·37
α-D-Galactose*	*c.* 2,920*s*	2,195*m*	*c.* 1·33	1,363*s*	1,132*s*	1·21	1,315*m*	1,036*m*	1·27
β-D-Galactose†	*c.* 2,900*s*	2,070*m* 2,055*m*	*c.* 1·38	1,387*s*	1,115*m*	1·24	1,340*s*	966*s*	1·38
β-D-Arabinose*	*c.* 2,900*s*	2,180*m*	*c.* 1·33	1,357*s*	1,115*s*	1·22	1,318*s*	1,035*s*	1·27
α-D-Xylose*	*c.* 2,870*s*	2,210*m*	*c.* 1·30	1,340*s*	1,065*s*	1·26	1,305*s*	975*s*	1·34

* Equatorial hydrogen on $C_{(1)}$.

† Axial hydrogen on $C_{(1)}$.

All spectra were measured in a Perkin Elmer 21 double-beam spectrometer having a sodium chloride prism. The samples were dispersed in pressed potassium chloride disks.

research. One of us (R. H. M.) thanks Glaxo, Ltd. for the award of a scholarship.

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[1] Stacey, Barker, Bourne, Moore, Weigel and Whiffen, Second U.N. Internat. Conf. Peaceful Uses of Atomic Energy, *P*/1466 (1958).
[2] Larnaudie, *C.R. Acad. Sci., Paris*, **235**, 154 (1952).
[3] Corey, Sneen, Danaher, Young and Routledge, *Chem. and Indust.*, 1294 (1954).
[4] Jones, Humphries, Herling and Dobriner, *J. Amer. Chem. Soc.*, **73**, 3215 (1951).
[5] Cole, Jones and Dobriner, *J. Amer. Chem. Soc.*, **74**, 5571 (1952).
[6] Lloyd and Dodgson, *Nature*, **184**, 548 (1959).
[7] Orr, *Biochim. Biophys. Acta*, **14**, 173 (1954).
[8] Mathews, *Nature*, **181**, 421 (1958).
[9] Steele and Whiffen, *Tetrahedron*, **3**, 181 (1958).

Ultra-Violet Spectra of Water Solutions

Recently, investigations of the ultra-violet absorption spectra of inorganic and organic ions in the wave-length range below 250 mμ have been made. At the shortest wave-lengths obtainable on the commercial instruments some interesting results were obtained. These show that the absorbances measured were not primarily due to the ions under examination, but resulted from a series of water absorption peaks which are strongly enhanced by the presence of solutes. The spectra were measured on a Beckman *DK*2 using 1-cm. silica cells, and one sample was reproduced on a 'Uvispek' with silica optics.

The spectrum of pure water at room temperature was found to be relatively weak (oscillator strength at 297° K. is $1{\cdot}6 \times 10^{-7}$) and to have three main peaks at 1880, 1890 and 1908 Å. The absorption is very dependent on temperature, as is shown in Fig. 1.

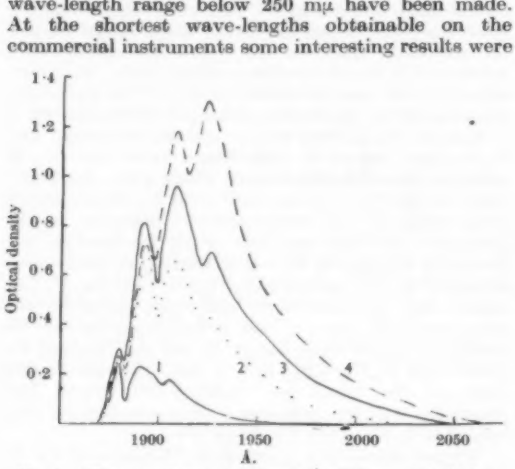

Fig. 1. Water spectrum at various temperatures. (1) 25° C.; (2) 91° C.; (3) 110° C.; (4) 155° C.

Fig. 2 illustrates the effect of dissolved solutes. A wide range of both inorganic and organic compounds was examined and the water peaks were always found. It is hoped to publish soon a more detailed paper on these observations, which show that the spectra of water solutions in the range 185–200 mμ are not true solute spectra, but an enhancement of the water peaks. It is probable that the series of water peaks may be due to a 'forbidden' transition to the triplet state for the water molecule. This may be induced by the electrostatic field strengths of the solutes.

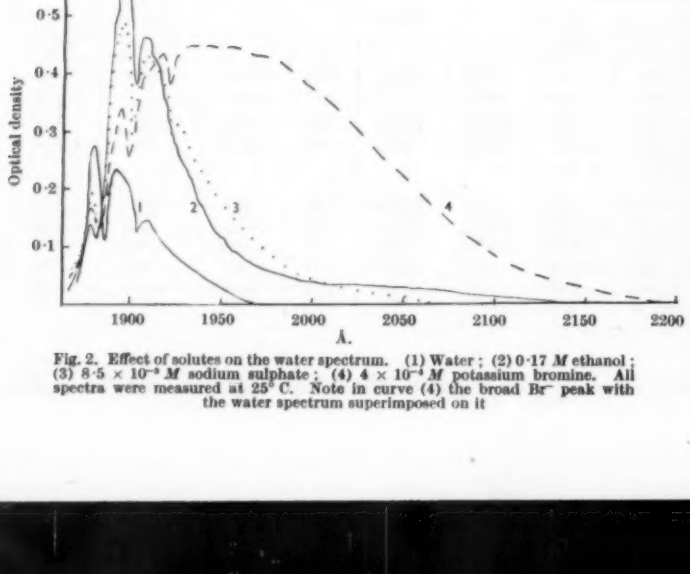

Fig. 2. Effect of solutes on the water spectrum. (1) Water; (2) 0·17 *M* ethanol; (3) $8{\cdot}5 \times 10^{-3}$ *M* sodium sulphate; (4) 4×10^{-4} *M* potassium bromine. All spectra were measured at 25° C. Note in curve (4) the broad Br^- peak with the water spectrum superimposed on it

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Sea-Water as the Primary Source of Sulphate in Tidal Swamp Soils from Sierra Leone

One of the main features of the chemical composition of the black glutinous muds of relatively high organic content in the Sierra Leone River Estuary is their high content of sulphide. Work carried out by the Rice Research Station at Rokupr on the Scarcies River, some 30 miles north of the Sierra Leone River estuary, suggests that the principal source of oxidizable sulphur in these estuarine soils is iron disulphide, or possibly some other polysulphide[1]. The presence of sulphide in marine muds has been ascribed by various authors to a number of causes. It may be produced by the way of bacterial decomposition of organic matter containing sulphur. On the other hand, it is known that certain bacteria in the presence of organic material can reduce sulphates to sulphides; this reaction probably accounts for the presence of ferrous sulphide in marine muds in many parts of the world.

Interest has been focused on the marginal swamp soils in Sierra Leone within recent years because of the possibility of using them for rice cultivation after empoldering. On drying, however, very low *p*H values are obtained, and toxic conditions inimical to rice cultivation are developed unless arrangements can be made to leach the acids and salts from the soil with fresh water. The part that the sulphur fraction plays in producing these conditions is not yet fully understood, but it would appear to be of some considerable importance and must be elucidated

before a satisfactory treatment for the soil can be worked out.

The results of a recent preliminary investigation into the composition of a tidal mud flat in the Sierra Leone River estuary suggests that a considerable proportion of the sulphur in these soils may possibly be derived from the overlying estuarine water. A series of mud samples from twenty-eight sites, arranged in four equidistant parallel rows, were examined for their organic contents, and in addition the chlorinity and sulphate content of the seepage water was determined. A sample of the estuarine water from the shore of the mud flat was also collected, and its chlorinity and sulphate content determined for comparative purposes.

The single values for chlorinity and sulphate obtained for the estuary water were 16·20 ‰ and 2·148 ‰ respectively. Qualitative determinations for sulphate and chloride carried out on the water from some fresh-water feeder streams within a few weeks of the estuarine determinations showed only a trace of either ion present. This suggests that the bulk of the sulphate in the estuarine water is derived from marine rather than terrestrial sources.

Table 1. Average Chlorinity and Sulphate Content of the Seepage Water compared with the Average Organic Content (Loss on Ignition) of the Mud Column for Each Row of the Sampling Pattern

Row	No. of samples	Cl (‰)	SO_4 (‰)	SO_4 per unit (gm.) Cl	Organic (loss on ignition) (per cent)
1	6	15·1	1·620	0·1073	21·65
2	7	16·7	2·064	0·1236	21·92
3	7	17·1	2·259	0·1321	23·08
4	8	17·1	2·257	0·1320	23·31

In Table 1, the average chlorinity and sulphate content of the seepage water has been compared with the average organic content (loss on ignition) of the mud column for each row of the sampling pattern. Table 1 reads downwards with increased distance from the shore of the mud flat. It will be seen that although the relationship between the organic content and the values for chlorinity and sulphate is not an exact one, it is sufficiently close to indicate that the horizontal gradients in the concentrations of the two constituents are probably due to differential absorption by the organic fraction and also, possibly, by the inorganic colloidal fraction of the mud.

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[1] Rice Research Station, Rokupr. Annual Report for 1957 (Crown Agents for Overseas Govts. and Admins., London, 1958).

Mechanism of the Electroreduction of Complexes of the Type $[M(NH_3)_5X]^{n+}$

The effect of various anions upon the reduction of $[Co(NH_3)_5X]^{2+}$ at the dropping mercury electrode was reported recently by Vlček and Kůta[1]. They explained the generation of the electrochemically active species in terms of a bimolecular substitution process that was proposed earlier[2] and has since been the subject of correspondence between Dr. Vlček and one of us (G. W. W.).

It was proposed[2] that cathode current efficiencies observed for the electrodeposition of nickel from liquid ammonia solutions of spin-free nickel (II) complexes are best interpreted in terms of an S_N2 ligand substitution process and that resultant translabilization is probably the rate-determining process. The communication by Vlček and Kůta prompts us to report more recent results in support of this interpretation.

Under conditions strictly analogous to those employed earlier[2], the cathode current efficiencies found for the electrolysis of liquid ammonia solutions of $[Co(NH_3)_6](NO_3)_3$, $[Co(NH_3)_5I](NO_3)_2$, and $[Co(NH_3)_5SCN](NO_3)_2$ are 24, 33, and 47 per cent, respectively. Thus, the trend in cathode current efficiency parallels the increasing *trans* effect, as would be predicted on the basis of the work of Watt and Hazlehurst[2]. Moreover, we have found evidence for the *in situ* generation of $[Co(NH_3)_5X]^{2+}$ by electrolysing $[Co(NH_3)_6]^{3+}$ in the presence of I^- and SCN^-; the observed cathode current efficiencies were 36 and 46 per cent, respectively. Within experimental error, these results are the same as those obtained with *a priori* incorporation of the I^- and SCN^- ligands.

A more detailed account of these experiments will be published elsewhere.

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[1] Vlček, A. A., and Kůta, J., *Nature*, **185**, 95 (1960).
[2] Watt, G. W., and Hazlehurst, D. A., *J. Electrochem. Soc.*, **106**, 117 (1959).

A Micro Gas Chromatograph

We were in need of a gas chromatograph for very small samples, and as a result of a recent paper by Martin[1], we began to construct a katharometer of very small bore. We finally succeeded in stretching a 10μ tungsten wire 40 mm. long along the axis of a copper tube of 0·4 mm. bore and also of a stainless tube of 0·3 mm. bore, the cell volume of the latter being a little less than 3 μl. It is possible to construct a detector of still smaller bore. We had only vague ideas as to the column to be used in connexion with this microdetector. The column tube must obviously have a bore comparable with that of the detector. To fill such a capillary with an ordinary support of powder form would be extremely difficult and would require a great deal of effort. We considered, therefore, a support of thin wire or rope form, or better still, the relatively large internal surface of the capillary alone. Then the work of Golay[2] came to our notice. We tried with success an internally coated empty capillary of 0·3 mm. bore. Two supports of wire or rope form are also very promising, namely, a 0·3-mm. simple copper wire with a few thorn-like protuberances on its surface to keep the wire coaxially inside a 0·5-mm. bore capillary, and a 0·5-mm. double coil of tungsten wire with its central mandrel.

Samples of size ranging from 10^{-4} to 10^{-5} ml. and sometimes 10^{-6} ml. have been injected with a high degree of reliability by means of a hypodermic needle. This holds a capillary ampoule—already opened or still closed—near its nozzle and a rubber stopper in its socket, through which a steel rod can be moved for displacing or crushing the ampoule.

The microdetector is as stable as an ordinary 4-mm. bore detector with a filament of the same dimensions and has a sensitivity of about 10^{-10} mole per c.c.

In a Golay column the liquid phase is a thin film, and it can be subjected to any reasonable programme of change of temperature, for it is in intimate contact with the tube wall, the temperature of which, in its turn, being exactly measurable, can be easily regulated according to the programme. This is done by an electric current through it and an air current blown against it. The change is rapid due to the low heat capacity and high conductivity of the tube. Its relatively high electrical resistance does not demand an inconveniently large current.

Tentative experiments made with a capillary column exposed to free access of air of a room kept at a constant temperature (20° C.) have given sufficiently reproducible chromatograms, the time required for the column temperature to fall from 100° to 20° C. being only about 2 min. in the unstirred air of the room.

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[1] Martin, A. J. P., "Vapour Phase Chromatography", 1, edit. by Desty, D. H. (Butterworths Scientific Publications, London, 1957).
[2] Golay, M. J. E., "Gas Chromatography", edit. by Coates, V. J., *et al.*, 1 (Academic Press, Inc., New York, 1958).

BIOCHEMISTRY

Condensation Product of Ethylenediamine with Noradrenaline or 3,4-Dihydroxymandelic Acid

It was found that the condensation reaction of ethylenediamine with adrenaline gave the main condensation product of noradrenaline as one of its by-products[1]. From this result, it was supposed that the side-chain of these two catechol amines could be destroyed during the condensation reaction and that the structure of the main condensation product of noradrenaline might be simpler than that of adrenaline. To investigate the structure of the main condensation product of noradrenaline, we examined further the condensation products of ethylenediamine with catechol and its derivatives which have a simpler structure than adrenaline or noradrenaline. For this purpose, 3,4-dihydroxymandelic acid (kindly provided by Dr. M. D. Armstrong) and catechol were used.

The condensation reaction of noradrenaline, 3,4-dihydroxymandelic acid or catechol with ethylenediamine and *iso*-butanol extraction of the condensates were carried out as reported by Weil-Malherbe and Bone[2]. Each *iso*-butanol extract was condensed and re-dissolved in a small amount of water.

The samples were first examined by paper chromatography using the following different solvents: (1) 5 per cent $Na_2HPO_4.12H_2O$ (ref. 1), (2) *n*-butanol-saturated phosphate buffer at *p*H 6·0 (ref. 3), (3) *n*-butanol/ethanol/5 per cent $Na_2HPO_4.12H_2O$ (50 : 25 : 30)[4], (4) *n*-butanol/acetic acid (100 : 15) saturated with water[2], and (5) methanol/concentrated ammonia (9 : 1)[5]. The paper strips were observed under ultra-violet light.

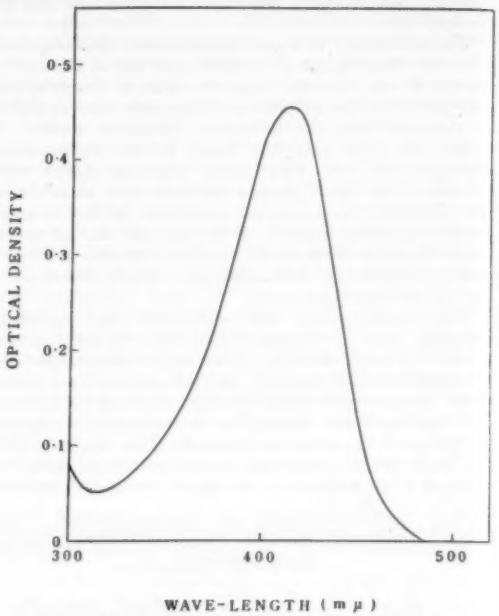

Fig. 1. Absorption spectrum of the main condensation product of ethylenediamine with noradrenaline, 3,4-dihydroxymandelic acid or catechol. Aqueous solution

Substances giving a bluish-green fluorescence, which were markedly dominant with each of noradrenaline, 3,4-dihydroxymandelic acid and catechol, had identical R_F values. The R_F values in the case of the five solvents were: (1) 0·03, (2) 0·20, (3) 0·72, (4) 0·64 and (5) 0·57 respectively.

Paper electrophoresis was then used to examine the products. Each sample was placed on the anode side of a paper strip moistened with 0·05 *M* phosphate buffer (*p*H 8·0) and 1 m.amp./cm. of constant current was applied in the dark. The main spot of each sample which fluoresced bluish-green showed the same rate of movement.

After the main condensation product of each sample was separated by paper chromatography using 5 per cent $Na_2HPO_4.12H_2O$ as solvent or by electrophoresis, their physico-chemical properties were studied.

The absorption spectra of aqueous extracts of these products were identical with each other and had $\lambda_{max.}$ at 414 mμ, as shown in Fig. 1, whereas that of the main condensation product of adrenaline showed $\lambda_{max.}$ at 410 mμ.

Fluorescence spectra of *iso*-butanol extracts and *p*H-fluorescence curves of aqueous extracts of these derivatives were identical with those of the main condensation product of ethylenediamine with noradrenaline, which has already been reported[1].

These results confirm the identity of the three bluish-green fluorescent substances. It was therefore supposed that the side-chain of noradrenaline, or of 3,4-dihydroxymandelic acid, might be severely damaged and still form the same condensation product with catechol. Thus, the chemical construction of the main condensation product of noradrenaline or 3,4-dihydroxymandelic acid is considered to be the

same as that of catechol, which was determined to be 1 : 2 : 3 : 4-tetrahydro-1 : 4 : 5 : 8-tetraaza-anthracene by Harley-Mason *et al.*[6].

Considering the chemical structure of the main condensation product of adrenaline, dihydro-3-hydroxy-1-methylpyrrolo-[4 : 5-*g*]-quinoxaline[6], the conjunction of methyl group is thought to have a protective effect on the side-chain during the condensation reaction.

After the above communication was submitted Drs. Joly and Nadeau discussed our earlier communication concerning the cleavage of the side-chain of catechol amines[1] (Joly, L. P., and Nadeau, G., *Nature*, **184**, 1483 (1959)). Their comments are almost answered by our second communication. However, the possibility of the stepwise cleavage of the side-chain of adrenaline, for example, through N-demethylation or α–β-cleavage, could not be excluded. This possibility remains for investigation.

We would like to correct here also a misprint in our communication entitled "Formation of Noradrenaline from Adrenaline by Rat Liver Mitochondria" (Yagi, K., and Nagatsu, T., *Nature*, **184**, 1316 (1959)). The first paragraph should read as follows. "By condensation with ethylenediamine, adrenaline gives the main condensate of noradrenaline with ethylenediamine as a side-product besides the main product of the reaction of adrenaline with ethylenediamine."

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[1] Yagi, K., and Nagatsu, T., *Nature*, **183**, 822 (1959).
[2] Weil-Malherbe, H., and Bone, A. D., *Biochem. J.*, **51**, 311 (1952).
[3] Nadeau, G., and Joly, L. P., *Nature*, **182**, 180 (1958).
[4] Yagi, K., Kondo, H., and Sumi, M., *J. Jap. Biochem. Soc.*, **27**, 777 (1956).
[5] Gray, I., and Young, J. G., *Clin. Chem.*, **3**, 239 (1957).
[6] Harley-Mason, J., and Laird, A. H., *Biochem. J.*, **69**, 59, *P* (1958).

Amide Nitrogen Content of Ovomucoid

THE usually accepted value for the amide nitrogen content of ovomucoid is 1·00 per cent[1] or 21 residues per molecular weight of 28,800 gm. (ref. 2). This value was obtained by acid hydrolysis of the protein followed by distillation of the ammonia from mildly alkaline solution. It is known, however, that ovomucoid contains hexosamine and that the latter is deaminated to a greater or lesser extent by the methods used. It is thus likely that the reported values for the amide nitrogen content of ovomucoid, and those of other glycoproteins which have been determined by similar procedures, are too high.

The hexosamine content of ovomucoid has been re-examined by the Cessi modification of the Elson–Morgan procedure (private communication), after hydrolysis of the protein at 100° under the following conditions. After hydrolysis in 2 *N* hydrochloric acid for periods of time of 2, 4, 6, 8, 12 and 24 hr., the amounts of hexosamine found in the protein were 12·6, 12·7, 14·0, 13·9, 13·9, and 11·9 per cent respectively. Hydrolysis in 4 *N* hydrochloric acid for 2, 4, 6, 8 and 12 hr. gave rise to 15·3, 15·0, 15·1, 15·5 and 14·7 per cent hexosamine. After 8 hr. hydrolysis in 4 *N* hydrochloric acid, 16·7 per cent hexosamine was found by the direct Elson–Morgan procedure, as described by Rondle and Morgan[3], compared with 15·5 per cent by the Cessi modification.

The total hexosamine plus amide nitrogen of ovomucoid was determined by steam distillation of the ammonia, under conditions whereby the amino-sugar is deaminated quantitatively[4], after hydrolysis of the protein in both 2 *N* and 4 *N* hydrochloric acid. In the former case, a maximum of 1·64 per cent amide nitrogen was reached after 1½ hr. which remained constant, and in the latter case 1·60 per cent.

Thus reasonable values for (i) the glucosamine + sialic acid content of ovomucoid would seem to be 15·2 per cent, or 24·4 residues per mole, and (ii) the glucosamine plus amide nitrogen of the order of 1·62 per cent or 33·3 residues per mole of ovomucoid. From this it might be deduced that the number of amide residues per mole of protein is about 9, and, indeed, direct estimation of the amide nitrogen by an independent method, and also titration curve results, tend to support this, when considered in the light of analytical data for the protein[1].

Direct estimation of the amide nitrogen content of the protein has been carried out by hydrolysis in 2 *N* hydrochloric acid for 3 hr. at 100° and the liberated ammonia precipitated by means of phosphotungstic acid at *p*H 3. The ammonia content of the precipitate was measured and found to correspond to 0·45 per cent expressed as the amide nitrogen content of the protein. This represents 9·3 residues per 28,800 gm. ovomucoid, in good agreement with the value found above.

Ovomucoid has been titrated in 0·2 *M* potassium chloride at 22° C. from *p*H 2 to *p*H 10. The experimental points obtained fit very closely to the Henderson–Hasselbach theoretical line obtained for 18 groups with *pK* 3·45, 11 groups with *pK* 4·9, 5 with *pK* 6·9, and 9 with *pK* 9·6. The five groups with *pK* 6·9, assuming they are imidazole groups, indicate the presence of five histidine residues, in good agreement with the value found by microbiological technique[1]. The lower two *pK* values, 3·45 and 4·9, involve 29 groups in all, which are assumed to be β-aspartyl, γ-glutamyl and the C-terminal phenylalanyl[5] carboxyl groups. This value, in conjunction with 9 amide groups, would indicate the presence in ovomucoid of 37 aspartic and glutamic acid residues, which is in fair agreement with the 41 residues determined experimentally[1], particularly since the carbohydrate in the protein may be bound through aspartic acid residues as it is in other glycoproteins[6]. This lower value for the amide nitrogen content makes clearer the reason for the acid isoelectric point of the protein.

The glucosamine in the protein is probably largely if not wholly N-acetylated, since hydrolysis of the protein in 2 *N* hydrochloric acid, followed by steam distillation of the fatty acids, and analysis by the method of Bueding and Yale[7] or by the gas chromatographic technique[8], indicated the presence of 24·2 and 24·7 acetyl groups by the respective methods per mole of ovomucoid. Three equivalents of formic acid were also found by the gas chromatographic technique; the origin of the formic acid is being further investigated.

It is possible that differences between some of our results and those of other workers are partly explicable in terms of the known heterogeneity of ovomucoid. The ovomucoid used was purchased from the Worthington Biochemical Sales Co., New Jersey (No. 01529), and had been prepared by the method of Lineweaver and Murray[2].

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[1] Lewis, J. C., Snell, N. S., Hirschmann, D. J., and Fraenkel-Conrat, H., *J. Biol. Chem.*, **186**, 23 (1950).
[2] Lineweaver, H., and Murray, C. W., *J. Biol. Chem.*, **171**, 565 (1947).
[3] Rondle, C. J. M., and Morgan, W. T. J., *Biochem. J.*, **61**, 586 (1955).
[4] Tracey, M. V., *Biochem. J.*, **52**, 265 (1952).
[5] Pénasse, L., Jutisz, M., Fromageot, C., and Fraenkel-Conrat, H., *Biochim. Biophys. Acta*, **9**, 551 (1952).
[6] Johansen, P., Marshall, R. D., and Neuberger, A., *Nature*, **181**, 1345 (1958). Jevons, F. R., *ibid.*, **181**, 1346 (1958). Cunningham, L. W., Cunningham, B. J., and Nuenke, R. B., *Biochim. Biophys. Acta*, **26**, 660 (1957). Rosevear, J. W., and Smith, E. L., *J. Amer. Chem. Soc.*, **80**, 250 (1958).
[7] Bueding, E., and Yale, H., *J. Biol. Chem.*, **193**, 411 (1951). Bueding, E., "Methods in Enzymology", **3**, 367 (1957).
[8] James, A. T., and Martin, A. J. P., *Biochem. J.*, **50**, 679 (1952).

Vitamin A Isomers in the Liver of Rats and Chicks

A recent communication reported the occurrence of all the 'unhindered' forms of vitamin A in fish-liver oils[1]. The isomers may be classified according to the rate of reaction with maleic anhydride as either 'fast'- or 'slow'-reacting[2,3]. Lambertsen and Brækkan[4] reported the presence of only small amounts of neovitamin A in fresh fish livers, as measured by the method of Robeson and Baxter[5]. The method only distinguishes between 'fast'- and 'slow'-reacting isomers[3]. At present our work with vitamin A deals only with these groups and not with the determination of the different isomers. The reported content of 14 per cent of *iso-a* (fast) + *iso-b* (slow) vitamin A in rat liver[1], however, prompts us to publish some of our findings not yet recorded.

We have found that fresh livers from rats contain very little 'slow'-reacting isomers provided that the animals were not fed appreciable amounts of such isomers. A few results obtained with chicks indicated similar conditions in this species (Table 1). The neovitamin A preparations tested were made by allowing a vitamin A concentrate containing 36 per cent neovitamin A to react with maleic anhydride in benzene solution for 48 hr. Excess maleic anhydride was removed by washing with water. The neoester was purified by chromatography on aluminium oxide; the alcohol was prepared by saponifying the ester. All preparations were diluted with arachis oil and given by dropper. Random samples of livers from sheep, cows, calves and pigs obtained from a slaughter-house gave varying results. It is possible that the animals had been fed vitamin preparations containing neovitamin A prior to slaughtering, and also that some isomerization had taken place afterwards (see below).

Table 1. Content of Neovitamin A ('Slow'-reacting Isomers) in the Liver of Rats and Chicks given Vitamin A Sources with Different Content of Such Isomers

Vitamin A source	Neovitamin A (per cent) in the liver of		
	in the oil	Rats	Chicks
Synthetic vitamin A acetate	0	3	
Synthetic vitamin A palmitate	4	0	
Synthetic vitamin A palmitate	6		2·5
Neovitamin A alcohol (DPI)	100	38	
Neovitamin A ester made from a concentrate	100	37	8
Neovitamin A alcohol made from the above ester	100	32	
Concentrate from which the above neovitamin A preparations were made	36	7	

It was further found that if homogenized livers from rats were stored at room temperatures appreciable amounts of 'slow'-reacting isomers appeared in 2–7 days. Storage at +4° C. and at lower temperatures produced only small amounts (Table 2).

Table 2. Percentage Content of 'Slow'-reacting Isomers of Vitamin A in Homogenized Rat Livers stored for Different Times at Different Temperatures. Content at Zero Time was Nil

Storage temperature	Days of storage						
	1	2	4	7	9	15	36
+ 30	5·0	13					
+ 18	1·3	3·7	8·1	14			
+ 4		0·6	0	0·6	0	1·9	
0		1·9	1·6	1·4	1·4	1·9	3·8
− 2		0	0	1·3	2·0	2·3	1·8
− 15				1·3		1·9	
− 30				1·8		1·6	

The results of the feeding experiments indicate that 'slow'-reacting isomers of vitamin A in the liver of rats may primarily be derived from the food. The experiments involving storage of homogenized rat livers prior to analysis show that isomerization readily takes place *in vitro*. These results indicate that the 'fast'-reacting isomers are the naturally occurring forms in the liver of rats and chicks.

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[1] Brown, P. S., Blum, W. P., and Stern, M. H., *Nature*, **184**, 1377 (1959).
[2] Robeson, C. D., Cawley, J. D., Weisler, L., Stern, M. H., Eddinger, C. C., and Chechak, A. J., *J. Amer. Chem. Soc.*, **77**, 4111 (1955).
[3] Plack, P. A., *Biochem. J.*, **64**, 56 (1956).
[4] Lambertsen, G., and Brækkan, O. R., "Biochemical Problems of Lipids", edit. by Popjak, G., and Le Breton, E., 56 (Butterworth, London, 1956).
[5] Robeson, C. D., and Baxter, J. G., *J. Amer. Chem. Soc.*, **69**, 136 (1947).

A Connexion between S—S Bond Breakage and Inactivation by Radiation of a Dry Enzyme

It has previously been established that the inactivation of a dry enzyme by ionizing radiation requires an ionization either within the enzyme molecule or very close to it[1]. It is known that the radiosensitivity can be varied by a factor of the order of magnitude of 2 by temperature[2], oxygen tension[3], and materials which surround the enzyme molecule in the dry state[4]. The mechanism for the inactivation is unknown.

Evidence that S—S bond breakage is involved in radiation inactivation in the dry state has been

obtained from work in which samples of the enzyme ribonuclease were treated with proteolytic enzymes and the resulting peptide fragments separated by paper electrophoresis followed by chromatography. As first shown by Hirs[5], native ribonuclease cannot be broken down into peptides by the action of trypsin unless the disulphide bonds are cleaved, thus unfolding the molecule. In Fig. 1 (left) is shown a 'fingerprint' of a sample of trypsin-digested bovine pancreatic ribonuclease (Armour) in which the S—S bonds had first been broken by reduction with thioglycollic acid and alkylation of the resulting SH groups with iodoacetic acid[6]. The electrophoresis (buffer: water/pyridine/glacial acetic acid, in the proportions 95 : 5 : 0·2, *p*H 6·5) was carried out along the horizontal axis, with the positively charged groups moving to the right. Descending chromatography (solvent: normal butyl alcohol/glacial acetic acid/water in the proportions 3 : 1 : 1 by volume) was carried out afterwards in the vertical direction. The peptide spots were developed by the usual ninhydrin reaction.

Fig. 1 (right) shows a 'fingerprint' of a sample of ribonuclease irradiated in vacuum with cobalt-60 gamma-rays to about 50 per cent of initial activity, and then treated with trypsin, without any chemical reagent being used to break S—S bonds. When an irradiated sample is treated with thioglycollic acid and iodoacetic acid before trypsinization, the broad smear representing the large undigested pieces disappears, and the 'fingerprint' looks exactly like Fig. 1 (left). A similar result is obtained when the digestion is carried out by both trypsin and chymotrypsin.

The remarkable agreement between the 'fingerprints' of the irradiated samples and those of unirradiated controls with the S—S bonds broken is strongly suggestive. It is possible that proteolytic digestion could occur either after S—S bond breakage, or after rupture of the polypeptide backbone. The latter possibility cannot at the moment be ruled out, but appears unlikely in view of the results of Alexander, Hamilton and Stacey[7], who have shown that no bonds in the polypeptide backbone are broken on irradiation of bovine serum albumin. Furthermore, several years ago, D. L. Aaronson at Yale (unpublished work) established that on irradiation of dry di- and tri-peptides, no intact amino-acids were released, indicating that the breakage of the peptide bond in the dry state is not a likely process. In this connexion, it might also be remembered that Gordy[8] found the same electron paramagnetic resonance spectrum for a number of irradiated proteins and for irradiated cystine.

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[1] Pollard, E. C., Guild, W. R., Hutchinson, F., and Setlow, R. B., "Prog. in Biophys.", **5**, 72 (1955).
[2] Pollard, E. C., Powell, W., and Reaume, S. H., *Proc. U.S. Nat. Acad. Sci.*, **38**, 173 (1952).
[3] Alexander, P., *Rad. Res.*, **6**, 653 (1957).
[4] Braams, R., Hutchinson, F., and Ray, D., *Nature*, **182**, 1506 (1958).
[5] Hirs, C. H. W., *J. Biol. Chem.*, **219**, 611 (1956).
[6] Sela, M., White, jun., F. H., and Anfinsen, C. B., *Biochim. Biophys. Acta*, **31**, 417 (1959).
[7] Alexander, P., Hamilton, L. D. G., and Stacey, K. A., *Nature*, **184**, 226 (1959).
[8] Gordy, W. R., Ard, W. B., and Shields, H., *Proc. U.S. Nat. Acad. Sci.*, **41**, 983 (1955).

X-Ray Diffraction Study of Denatured Ferritin and Apoferritin

THE powder diffraction diagrams of both denatured ferritin and apoferritin have been compared. The observed differences between these diagrams indicate the probable arrangement of micelles in the structure of denatured ferritin.

The preparations of denatured ferritin and denatured apoferritin were examined by the X-ray powder diffraction method. Cobalt $K\alpha$ radiation monochromatized by filtration was employed. The photographs of samples dried over phosphorus pentoxide were obtained in a flat-film camera. The distance between the sample and the film varied from 4 to 10 cm. The diffractions corresponding to interplanar spacings of 2·3–90 Å. were registered.

Ferritin was isolated from horse spleen by Granick's modification[1] of Laufberger's method. Apoferritin was prepared by the method described by Behrens and Taubert[2].

Both ferritin and apoferritin were denatured by equal denaturing agents. Denaturation by heat, drying, 6 *M* urea, 5 *N* and 1 *N* hydrochloric acid and 1 *N* sodium hydroxide was used.

When ferritin is denatured by 5 *N* hydrochloric acid and 1 *N* sodium hydroxide the ferric micelles are freed from the protein part of ferritin and denatured apoferritin results[3,4].

The diffraction data of non-oriented preparations of denatured apoferritin are given in Table 1,*A*. The diagrams of denatured apoferritin are identical with those of other denatured corpuscular proteins for which the arrangement of polypeptide chains in pleated sheet β-configuration[5] is accepted.

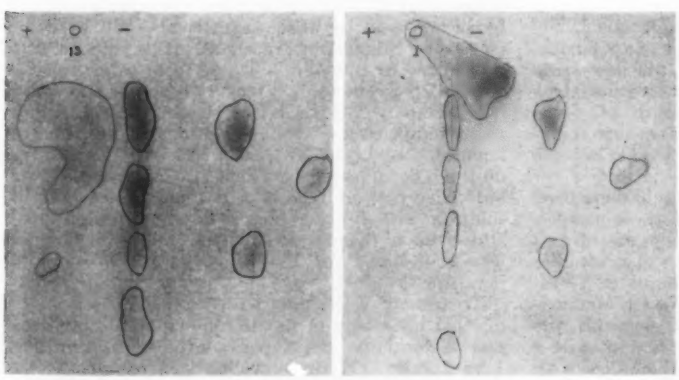

Fig. 1. Left: 'Fingerprint' of ribonuclease with disulphide bonds reduced and then digested with trypsin. Right: 'Fingerprint' of ribonuclease given 20 megarads of γ-radiation and then digested with trypsin

Table 1

n	A			B			C	
	d (Å.)	hkl	I	d (Å.)	hkl	I	d (Å.)	I
1				42·4	111	vs		
2				26·4	022	s		
3				18·6	004	m		
4	10·8	001	vs	11·2		s	10·0	vs
5							[7·7]	w
6				[6·3]		m	[6·9]	m
7							[5·6]	s
8	4·64	200	s	[4·67]		s		
9							[4·0]	w
10	3·70	210, 020	w	[3·3]		w	[3·7]	vw
11	2·74	004	m					
12				[2·5]		vw	[2·5]	vvw

The values in square brackets correspond to diffuse lines which were difficult to measure.
A, diffraction data of denatured apoferritin; *B*, diffraction data of denatured ferritin; *C*, diffraction data of the content of ferric micelles precipitated during alkalic hydrolysis of ferritin.

The diffraction diagrams of denatured ferritin (Table 1, *B*) contain several sharp low-angle diffractions (for $d > 15$ Å.) and diffuse diffractions in the wide-angle region (for $d < 15$ Å.). These diffuse lines correspond partly to the diffractions of denatured apoferritin (diffractions 4, 8 and 10), partly to the internal structure of ferric micelles of ferritin (diffractions 4, 6, 10 and 12). Table 1, *C* gives the diffraction results of the precipitate of the content of ferric micelles produced during alkaline hydrolysis of ferritin lasting 12 hr. In spite of the presence of rather diffuse diffraction lines in the diagrams of both preparations, the diffractions 6, 10 and 12 of denatured ferritin can be identified as those belonging to the internal structure of ferric micelles.

Low-angle diffractions of denatured ferritin (diffractions 1, 2 and 3) correspond to the mutual arrangement of two components of ferritin—protein part and ferric micelles. They were indexed on the basis of a cubic lattice having an edge $a = 74{\cdot}2$ Å. The type of the lattice could not be estimated uniquely for the number of diffractions found was too small. The arrangement of ferric micelles in the face-centred cubic lattice (the diameter of micelles is then 52·5 Å.) or the arrangement of the sub-units of ferric micelles in the cubic lattice of diamond-type structure (the diameter of sub-units is 32 Å. and that of micelles is then 64 Å.) are the most probable ones.

In both cases the spaces between the micelles or their sub-units are filled with polypeptide chains in β-configuration.

Every preparation of ferritin contains about 20–25 per cent apoferritin[5]. If the content of apoferritin in the denatured sample is increased, the low-angle diffractions (1, 2 and 3) and the diffuse diffractions corresponding to the internal structure of ferric micelles (6, 12) weaken and the diffractions corresponding to the denatured apoferritin (8, 11) become sharper and more intensive.

A model of the structural arrangement of denatured ferritin is proposed on the basis of these results. The structure of denatured ferritin consists of two types of crystalline regions. One type of crystallite contains only unfolded polypeptide chains in β-configuration—it has the structure of denatured apoferritin. The second type of crystallite contains the ferric micelles arranged in the cubic lattice ($a = 74{\cdot}2$ Å.). The spaces between micelles are then filled with the polypeptide chains in β-configuration; their orientation with regard to the axes of the lattice of micelles could not be estimated. The mutual orientation of these two types of crystalline regions is arbitrary. The regions of non-arranged components of ferritin can occur between them. The dimensions of crystalline regions are about 10^{-5}–10^{-6} cm.

Further experimental results will be necessary in order to solve definitively the arrangement of ferric micelles in denatured ferritin.

It was found that when ferritin is denatured by agents which do not free the iron from it, the ferric micelles remain in the structure of denatured ferritin and give rise to low-angle diffractions. On the other hand, the structure of denatured apoferritin is identical with that of other denatured corpuscular proteins characterized by the β-configuration of polypeptide chains.

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[1] Granick, S., *J. Biol. Chem.*, **146**, 451 (1942).
[2] Behrens, M., and Taubert, M., *Hoppe-Seylers Z. physiol. Chem.*, **290**, 156 (1952).
[3] Bénézech, C., and Magnan de Bornier, B., *Bull. Soc. chim. biol.*, **38**, 661 (1956).
[4] Granick, S., and Hahn, P. F., *J. Biol. Chem.*, **155**, 661 (1944).
[5] Pauling, L., and Corey, R. B., *Proc. U.S. Nat. Acad. Sci.*, **37**, 729 (1951).
[6] Rothen, A., *J. Biol. Chem.*, **152**, 679 (1944).

Liberation of Hexuronic Acid, Hexosamine and Hydroxyproline from Steer Hide by Stem Bromelain

Stem bromelain, an enzyme mixture, consisting primarily of plant proteases, with limited amounts of phosphatases, and amylase (Heinicke and Gortner[1]) was assayed for its action on the mucopolysaccharides, mucoproteins, and collagen of steer hide. Mucopolysaccharides and mucoproteins are found in ground substance, basement membrane, sheaths and layers of the hair follicle. Collagen is found mainly in fibrous elements of hide.

The liberation of hexuronic acid and hexosamine was used as an index of the enzymatic hydrolysis of mucopolysaccharides and mucoproteins, while the liberation of hydroxyproline was used as an index of the hydrolysis of collagen.

The hexosamine, hexuronic acid, and hydroxyproline content was determined for samples of freshly flayed steer hide (see Table 1). Hexosamine was determined by Anastassiadis and Common's method[2], hexuronic acid by Dische's method[3], and hydroxyproline by Stigemann's method[4]. Hide squares, 2 in. × 2 in., were soaked in a 4·5 per cent saline solution with 0·1 per cent sodium trichlorophenate added as a disinfectant. The period of soaking was 12 hr. at room temperature. Determinations of hexosamine, hexuronic acid, and hydroxyproline were carried out on the hide squares (see Table 1). After soaking, the hide squares were placed in a 1 per cent bromelain solution with 0·1 per cent sodium trichlorophenate added as a disinfectant. The bromelain solution was maintained for 12–17 hr., at pH 6–6·8, temperature 30–35° C.

Treatment of the hide with bromelain indicated a marked liberation of hexuronic acid (22·3 per cent) and hexosamine (24·5 per cent), an approximate 1 to 1 ratio. Hydroxyproline was released to the extent of 8·4 per cent. Associated with the liberation of the hexosamine and hexuronic acid was a depilatory effect on the steer hide. The hair was found to be

Table 1. Alterations of Hexosamine, Hexuronic Acid and Hydroxyproline Content of Steer Hide

	Hexosamine	Hexuronic acid	Hydroxy-proline
Control (γ/gm.)	2,390	1,028	13,650
Untreated hide (γ/gm.)	2,530	1,173	13,600
Hide after soak (γ/gm.)	2,501	1,110	12,680
Hide after bromelain (γ/gm.)	1,888	862	11,610
Liberated by bromelain (γ/gm.)	613	248	1,070
Percentage liberated by bromelain	24·5	22·3	8·4

loosened from the hair follicle. By applying a slight scraping action, the hair was completely removed from the squares. Frozen sections of the unhaired hide squares were cut and stained with thionin. Microscopic examination revealed: (1) complete removal of hair from the hair follicle; (2) removal of the epidermis from the corium; (3) fibre bundles of the corium appeared clear and sharp with marked increased spacings between the fibre bundles.

Further results of the work will be published at a later date. The bromelain powder was kindly supplied by the Hawaiian Pineapple Co., Ltd., San Jose, California.

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[1] Heinicke, R., and Gortner, W., *Econ. Bot.*, **2**, 225 (1957).
[2] Anastassiadis, P., and Common, P., *Canad. J. Biochem. and Physiol.*, **36**, 413 (1958).
[3] Dische, Z., *J. Biol. Chem.*, **167**, 189 (1947).
[4] Stigemann, H., *Z. physiol. Chem.*, **311**, 41 (1958).

ANIMAL PHYSIOLOGY

Some Effects of Different Tensions of Oxygen on the Respiration and Growth of *L*-Strain Fibroblasts

A considerable amount of work has been carried out in this Laboratory concerning the effects of various changes in the physiological components of the gas phase upon the respiration of living cells.

Following up a suggestion by Osgood[1] that the growth of human leucocytes might be dependent upon oxygen tension, Kieler[2] made a study of the metabolism of both normal and leukæmic leucocytes under various concentrations of oxygen. He observed that although the oxygen consumption of normal cells remained unaffected by the differing conditions in the surrounding gas phase, leukæmic leucocytes showed a decrease in the amount of oxygen taken up in both high and low oxygen tensions. The optimum gas[2] phase for the respiration of the malignant cells proved to be that containing 10 per cent oxygen and under these conditions the consumption of oxygen resembled that of normal cells.

Kieler[3] also carried out similar experiments with Yoshida rat ascites tumour cells and again an inhibition of oxygen consumption occurred with both high and low oxygen tensions. In this case the optimal tension of oxygen lay between 15 and 20 per cent of the gas phase.

It was decided to repeat such experiments with cells of the 'non-malignant' subline of *L*-strain mouse fibroblasts. The cells were cultured in suspension

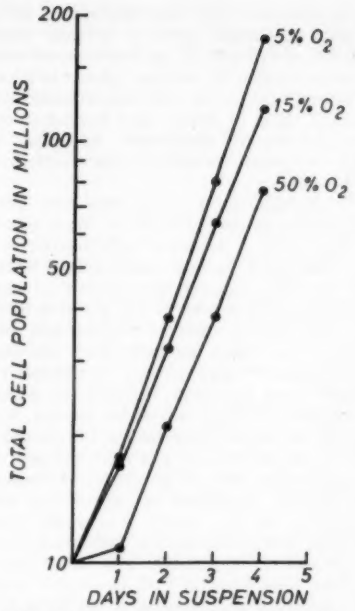

Fig. 1. Effect of 5, 15 and 50 per cent oxygen upon the growth of *L*-strain fibroblasts in suspension culture

using a medium of horse serum, fowl embryo extract and Tyrode solution and were stirred by means of a magnetic stirrer. The Cartesian diver technique was used to measure respiration, and nine experiments were carried out in this manner. Since, inevitably, the cells were not always in identical stages of growth and metabolism, the relative values of oxygen consumption were compared in each case instead of the absolute values.

The results can be seen in Table 1; it is clear that there is no significant difference in the effects of concentrations of 10, 15, 20 and 50 per cent oxygen in the gas phase. There is, however, a slight decrease in the oxygen consumption where only a 5 per cent oxygen tension is present, but this might be expected in normal cells. These results are comparable to those found with the normal leucocytes of Kieler's experiments, and are therefore in agreement

Table 1. Effect of 5, 10, 15, 20 and 50 per cent Oxygen Tension upon the Respiration of *L*-Strain Fibroblast Cells

Oxygen consumption per cell per hr. $\mu l. \times 10^{-6}$

Experiment	5% O_2 95% N_2	10% O_2 90% N_2	15% O_2 85% N_2	20% O_2 80% N_2	50% O_2 50% N_2
1	2·4	2·7	3·3		
2	3·46	3·65	3·4		
3		3·8	3·19	2·17	
4			2·8	2·85	2·92
5	3·9		3·7		3·93
6			4·55		4·45
7		3·95	4·7		
8	2·75		4·4		4·45
9	3·83		3·93	3·99	3·63

Relative values of oxygen consumption using values at 15 per cent oxygen as a standard 100

Experiment					
1	73	82	100		
2	102	107	100		
3		119	100	99	
4			100	102	104
5	103		100		106
6			100		98
7		84	100		
8	63		100	107	
9	97		100	101	91
Average of total	88	98	100	101	100

with the hypothesis that the respiration of normal cells is not dependent upon a certain tension of oxygen in the gas phase. If, as it seems, an independence towards oxygen in the gas phase is a criterion of the 'normal' cell, then this experiment also substantiates the claim of Philips and Feldhaus[4] that this particular sub-line of fibroblasts had lost, or was losing, the malignant metabolic characteristics of the original *L*-strain.

While culturing the *L*-strain sub-line, particular note was made of the growth of these cells under various tensions of oxygen. (It must be noted, however, that since carbon dioxide had been found necessary for the logarithmic growth of such suspension cultures, 2 per cent of this gas was introduced with the different amounts of oxygen and nitrogen.) Cell counts were taken at daily intervals and from these figures growth curves could be obtained. In all the cultures approximately logarithmic growth was maintained after a slight initial lag period, but there appeared to be some stimulation of growth in the cells with a gas phase of 5 per cent oxygen, in comparison with those with 15 per cent. A tension of 50 per cent oxygen produced an initial lag period of approximately 24 hr., which was much more pronounced than in the cells grown with lower tensions of oxygen; it was not cytocidal. The latter observation is in conflict with that of Cooper *et al.*[5], who found that the growth of suspensions of rabbit kidney cells was dependent upon different tensions of oxygen, and also observed that 25–30 per cent oxygen was cytocidal; 50 per cent oxygen was not tested.

One can conclude that growth, or cell multiplication, must be more dependent upon differences in oxygen tension than is the process of respiration. Presumably one, or more, of the mechanisms concerned in cell multiplication is highly sensitive to changes in the concentration of oxygen.

I wish to thank Dr. Jørgen Kieler for his assistance and guidance in this work which was carried out at the Fibiger Laboratory, Copenhagen. I also wish to acknowledge the financial support of the Churchill Foundation, the Rask Ørsted Foundation, the Anders Hasselbalch Anti-Leukæmia Fund, King Christian X Foundation, Einar Willumsen's Memorial Fund and the Leukæmia Society Inc.

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[1] Osgood, E. E., and Brooke, J. H., *Blood*, **10**, 1010 (1955). Osgood, E. E., and Krippaehne, M. L., *Exp. Cell. Res.*, **9**, 116 (1955).
[2] Kieler, J., "Cultivation of Leukemic Cells in the Cartesian Diver" in "The Leukemias: Etiology, Pathophysiology, and Treatment" (Acad. Press, New York, 1957).
[3] Kieler, J., "Studies on Oxidative Metabolism of Yoshida Ascites Tumour Cells", Seventh Internat. Cancer Congress, London (1958).
[4] Philips, H. F., and Feldhaus, R. F., *Proc. Soc. Exp. Biol. and Med.*, **92**, 478 (1956).
[5] Cooper, P. D., Burt, A. M., and Wilson, J. M., *Nature*, **182**, 1508 (1958).

Calcium and Contractility in Depolarized Smooth Muscle

It has been postulated that calcium ions act as the link between depolarization and contraction in cardiac[1] and skeletal muscle[2]. This hypothesis is based on the results of experiments relating the effect of depolarization on contraction with varying concentrations of calcium ions. The present work indicates

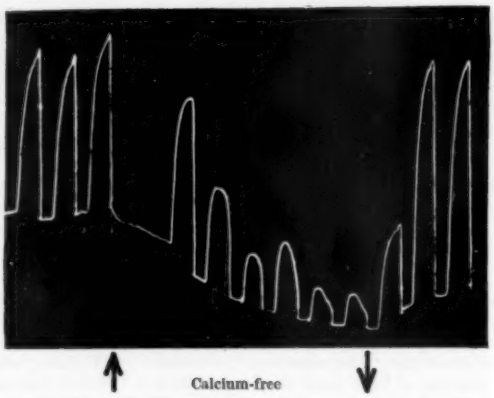

Fig. 1. Record of the isotonic contractions to acetylcholine. 1 μgm./ml. administered for 30 sec. every 2 min. to a strip of rabbit ileal longitudinal muscle suspended in potassium sulphate–Ringer. This was replaced between ↑ and ↓ by calcium-free potassium sulphate–Ringer which caused the acetylcholine responses to diminish gradually. This was in contrast to the rapid recovery which was significant when acetylcholine was added 15 sec. after the re-addition of calcium at ↓ and complete 2 min. later

that calcium ions are essential for contractility even in 'permanently' depolarized muscle.

As previously described, isolated smooth muscle preparations of rabbit and guinea pig ilea, rat uterus and chick amnion still contracted with acetylcholine, although already fully depolarized by immersion in isotonic potassium sulphate–Ringer solution[3]. It was found that the removal of calcium ions from the potassium sulphate–Ringer caused a reversible loss of response to acetylcholine. Thin preparations of muscle exhibited this effect more quickly than thick preparations and so strips of longitudinal muscle of rabbit ilea[4] were used in the majority of these experiments. The gradual reduction of the response to acetylcholine of a preparation exposed to calcium-free potassium–Ringer is shown in Fig. 1. After 10–12 min. the contractions were almost negligible compared with the initial responses. The quick recovery, which was significant at 15 sec. and complete at 2 min., after calcium was re-added to the bathing fluid suggests that the effect is an extracellular one. Trisodium or magnesium edetate 5 mgm./ml., when added 2 min. previously to the potassium sulphate–Ringer containing calcium ions, caused a severe though not complete reduction in the response to acetylcholine with a very slow recovery (20 min.) on washing out the chelating agent. In contrast, application of a similar concentration of edetate already complexed with calcium only slightly and transiently reduced the response of the tissue to acetylcholine.

The rather gradual development of the effects of the loss of calcium from the bathing fluid was probably due (*a*) to the slow diffusion outwards of calcium contained intra- or extra-cellularly within the muscle, for it was found that longitudinal muscle strips were affected more quickly than the thicker whole segments of rabbit ilea, and (*b*) to the presence of calcium as an impurity in the analytical grade reagents used in making up the calcium-free bathing fluid. A quicker and more complete loss of response to acetylcholine was obtained when trisodium or magnesium edetate (less than 10 mgm./l.), equivalent to the maximum limits of impurity of calcium in the reagents used, was added to the nominally calcium-free bathing

fluid. As only minute amounts of calcium are needed to exert an effect on frog nerve fibres[6] this result supports (*b*). However, as there was still a delay of approximately 10 min. before the muscle failed completely to respond to acetylcholine, the possibility (*a*) cannot be ignored.

These results show that the presence of calcium in the bathing fluid is necessary for the contraction with acetylcholine of isolated smooth muscle already depolarized by potassium sulphate. They tend to confirm the importance of calcium ions at the membrane. It may be that in smooth muscle acetylcholine increases the membrane permeability to calcium ions as well as to monovalent ions[6]. It has already been shown that in frog skeletal muscle there is a marked increase in calcium influx during activity[7]. However, the present results do not exclude the possibility that the presence of calcium is essential only within the cell for the activation of the contractile elements.

More recent experiments show that acetylcholine markedly increases the uptake of calcium-45 in depolarized smooth muscle.

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[1] Niedergerke, R., *Experientia*, **15**, 128 (1959). Weidmann, S., *ibid.*, **15**, 128 (1959).
[2] Frank, G. B., *Nature*, **182**, 1800 (1958).
[3] Evans, D. H. L., Schild, H. O., and Thesleff, S., *J. Physiol.*, **143**, 474 (1958).
[4] Ambache, N., *J. Physiol.*, **125**, 53*P* (1954).
[5] Frankenhauser, B., *J. Physiol.*, **137**, 245 (1957).
[6] Durbin, R., and Jenkinson, D. H., *J. Physiol.*, **148**, 68*P* (1959).
[7] Bianchi, C. P., and Shanes, A. M., *J. Gen. Physiol.*, **42**, 803 (1959).

Effects of Non-Ionic Surfactants upon Animal Tissues

In a previous communication[1] it has been reported that non-ionic surfactants produce a marked effect upon living plants. Setala *et al.*[2] have investigated the tumour-promoting capacity of certain non-ionic surfactants ('Span' and 'Tween') on mice, and these workers have indicated the need for further investigation of the co-carcinogenic properties of such substances.

The purpose of this communication is to report further studies involving the following animal tissues—gonads, liver and kidney. These studies reveal that very marked effects result from the application of some non-ionic surfactants upon different animal tissues, and that the effects vary, apparently according to the surfactant used and, to a greater degree, according to the kind of tissue to which it is applied.

In the course of this work the following surfactants were used: 'Pluronic *L*-61' (polypropylene glycolpolyoxyethylene condensate): '*G* 8416-*P*' (polyoxyethylene sorbitan esters of mixed fatty acid residues); 'Tergitol' (alkyl phenyl ether of polyethylene glycol) and 'Triton *X*-100' (alkylated aryl polyether alcohol).

The first experiments were done with '*G* 8416-*P*' and the choice of the other surfactants was dictated by their non-possession of a fatty acid moiety. The animals treated were rats and mice of both sexes and of two age-groups, pre-pubertal and post-pubertal.

The gonads were treated in the following manner. Ovaries of both age-classes were exposed by surgery and the surfactants delivered by means of an 'Agla' micro-syringe. Controls were either left untreated or were operated upon and the gonads touched by the same instrument as used for the application of the surfactant, or they were treated with control substances, for example, paraffin (B.P.) and oleic acid. Each experiment was performed using a group of at least three animals in each treatment. A group of 8 sexually immature female mice (average weight 7 gm.) was treated 5 times weekly with subcutaneous injections of a 0·01 per cent solution of 'Triton *X*-100'. The onset of œstrus did not differ from a control group of the same age. Two animals were killed after 7 weeks of treatment; the remaining 2 groups of 3 animals each were killed after 8 and 9 weeks respectively. No abnormal changes could be observed on the ovaries.

The testes of sexually mature animals were treated either by repeated external application of the surfactant upon the scrotum over an extended time period, or by surgically exposing the testes and applying known quantities directly upon the organ by means of the micro-syringe.

The kidneys and liver of other animals were likewise treated once after surgical exposure of the organ. Most of the animals treated on these organs died soon after application so no conclusive results are yet available. Experiments on these organs are being repeated employing lower concentrations of surfactants.

A distinct effect was obtained by treating ovarian tissue with the two surfactants '*G* 8416-*P*' and 'Triton *X*-100' (Table 1). Histological examination of ovarian tissue treated with 'Triton *X*-100', paraffin and oleic acid revealed that the increased tissue activity of the 'Triton *X*-100'-treated tissue is measurable by an evident increased number of Graaffian follicles and an increase in ovarian stroma. Furthermore, an increase in the frequency of occurrence and in size of cystic spaces and of haemorrhages obtained in the 'Triton' group. When the amounts of the surfactants were increased, atrophy of the gonads resulted.

Our observations on non-ionic effects upon testes seem to indicate that a regression can be obtained by using 'Triton *X*-100' and '*G* 8416-*P*', since an extensive regression resulted not only when testes were treated directly (operation) but also in mice

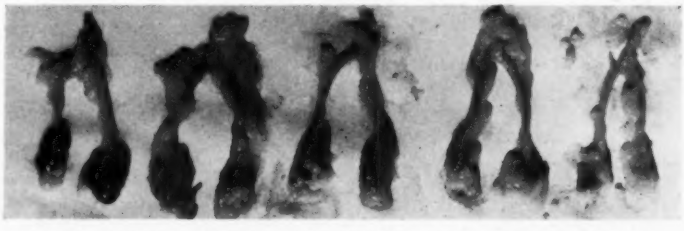

Fig. 1. The effect on sexually mature rat ovaries 8 weeks after a single direct application of 0·10 ml. of a 25 per cent solution of 'Triton *X*-100'. The ovarian fat has been removed from the two pairs of ovaries at the right to show more clearly the hæmatomas-like enlargement

Table 1. Summary of Ovarian Experiments

Organ treated	Species and number of animals	Surfactant used	Duration of experiment	Response
Ovary (sexually mature)	Rat (5)	'Triton *X*-100', 0·01 ml., undiluted	6 weeks	No apparent macroscopic difference
	Rat (5)	Control oleic acid, 0·01 ml. undiluted	6 weeks	No apparent macroscopic difference
			9 weeks	No apparent macroscopic difference
	Rat (5)	Control, paraffin (B.P.) 0·01 ml. undiluted	6 weeks	No apparent macroscopic difference
			9 weeks	No apparent macroscopic difference
	Rat (5)	Control, untreated	6 weeks	No apparent macroscopic difference
			9 weeks	No apparent macroscopic difference
Ovary (sexually mature)	Rat (5)	'Triton *X*-100', 0·10 ml. of 25 per cent solution	8 weeks	Ovaries enlarged and practically appearing as large hæmatomas (Fig. 1)
	Rat (7)	'Triton *X*-100', 0·10 ml. of 50 per cent solution	5 weeks	Two animals had large hæmatomas on both ovaries. One animal had a large colourless cyst on one ovary. The remaining four animals showed no macroscopic change
	Rat (7)	'Triton *X*-100', 0·10 ml. of 20 per cent solution	5 weeks	Three animals showed large hæmatomas on both ovaries. One animal showed cystic degeneration in both ovaries. The other three animals showed no apparent change
	Rat (7)	Control (touch)	5 weeks	No apparent macroscopic change
	Rat (7)	Control (untreated)	5 weeks	No apparent macroscopic change
Ovary (sexually mature)	Rat (5)	'Pluronic *L*-61', 0·01 ml. undiluted	2 weeks	Two rats killed showed no apparent macroscopic change
			5 weeks*	One animal killed. No apparent macroscopic change
	Rat (5)	Control, paraffin (B.P.), 0·01 ml. undiluted	9 weeks	No apparent macroscopic change
	Rat (5)	Control, oleic acid, 0·01 ml. undiluted	9 weeks	No apparent macroscopic change
	Rat (5)	Control, untreated	9 weeks	No apparent macroscopic change
Ovary (sexually immature) 4 weeks old	Rat (9)	'Triton *X*-100', both ovaries painted with 0·1 ml. of 10 per cent solution	1 week	2 rats killed†
			2 weeks	2 rats killed†
			3 weeks	2 rats killed†
			4 weeks	1 rat killed†
			6 weeks	2 rats killed. One ovary of one animal showed a large hæmatoma; no macroscopic changes observed in the other ovaries
	Rat (7)	Control, both ovaries touched: no surfactant	1 week	2 rats killed. No apparent macroscopic change
			2 weeks	2 rats killed. No apparent macroscopic change
			3 weeks	1 rat killed. No apparent macroscopic change
			4 weeks	1 rat killed. No apparent macroscopic change
			6 weeks	1 rat killed. No apparent macroscopic change
Ovary (sexually immature, average weight, 7 gm.)	Mouse (8)	'Triton *X*-100', 0·01 per cent solution injected subcutaneously five times per week	7 weeks	2 mice killed‡
			8 weeks	3 mice killed‡
			9 weeks	3 mice killed‡

* After this date the remaining two animals died accidentally. Therefore, no comparison at the 9-week level can be made.
† No apparent macroscopic difference in ovary; or of time of opening of the vagina or of the time of onset of œstrus.
‡ Onset of œstrus normal. No abnormal tissue changes noted.

when they were painted externally (via scrotum) twice weekly for a period of three months and killed four weeks later.

No effects were obtained by treating rats in this way, and this may be attributable to differences in the scrotal skin texture. No macroscopic changes were observed in the scrotal tissue of rats or mice after this prolonged treatment. Atrophy of rat testes attended direct application of undiluted 'Triton *X*-100'. No effects were obtained by direct application of 'Pluronic *L*-61', 'Tergitol', oleic acid or paraffin.

Investigations are also in progress to assess the effect of non-ionics on other animal tissues, especially as our experiments seem to indicate that there is a large variation in response by different tissues.

We wish to thank Dr. L. N. Loomis and Mr. L. Whitlock, chief laboratory technician, both of the School of Veterinary Science, University of Sydney, for their kind help in preparing and assessing the histological preparations.

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[1] Goldhammer, H., *Nature*, **178**, 1286 (1956).
[2] Setala, K., Holsti, P., Lundbom, S., and Setala, H., Proc. Second Int. Conf. concerning the Biochemical Problems of Lipids (Butterworths Scientific Publications, London, 1955).

Effect of Electrical Stimulation on a Muscle Fibre Model in the Presence of Adenosine Triphosphate

The mechanism linking the excitation of the plasma membrane of muscle fibres with the contraction of their myofibrils is a matter of great interest to many physiologists[1-3]. The present communication is concerned with the positive effect of the electrical stimulation on glycerinated muscle fibres[4] under specified conditions, suggesting a newer approach to the clarification of the above problem.

Fig. 1 shows that the electrical stimulation produces a slight but a definite enhancement, sometimes up to 20 per cent, on the rate of adenosine triphosphate-induced contraction of glycerinated fibres, though, as is well known, it has no effect whatever on the fibres in the absence of adenosine triphosphate. This phenomenon occurs if fresh fibres (within one month after the preparation) are used, and the enhancement can be observed in about 50 per cent of fibre bundles prepared from psoas muscles of the same rabbit, though the percentage depends upon the stage after the preparation. There exist two types of enhancement: one is without latency (Fig. 1), and the other with it. In the latter type the recovery after arresting the stimulation is not clear, while in the former the recovery is fairly complete. The former type of the enhancement is usually found in fresh preparations,

and the latter in relatively aged ones. A fatigue phenomenon due to continuous repetition by stimulation sets in. If the stimulation is given to muscle fibres in the stage of spontaneous relaxation after the shortening stage, the relaxation is inhibited or is converted into contraction (Fig. 2, b_1).

It is interesting that the effect of the stimulation tends to be increased by the addition of acetylcholinesterase inhibitor (prostigmine) (Fig. 2, *a*), and to be inhibited by the addition of 5×10^{-3} *M* atropine (Fig. 2, b_1 and b_2). *d*-Tubocurarine did not show any influence on this effect.

In connexion with these results, several suggestive effects were found: (1) If the fresh fibre bundles are used, their adenosine triphosphate-contraction is enhanced by the addition of small amounts of acetylcholine (10^{-7}–2×10^{-3} *M*) in the similar grade to the case of the electrical enhancement mentioned above (Fig. 2, *c*). (2) When the electrical enhancement is recognized, an acetylcholine-like substance can be demonstrated in the bath solution in which the fibre bundles have been immersed and stimulated electrically, by the bioassay using leech muscle. (3) If the supernatant obtained by centrifugation at 1,500*g* of the homogenate of the freshly prepared glycerinated muscle is stimulated electrically under conditions similar to the case of the above electrical enhancement, an acetylcholine-like substance is also found in the reaction mixture. Furthermore, the preliminary result has demonstrated that, if the ultracentrifugal fraction from 10,000*g* to 80,000*g* of the muscle homogenate is stimulated electrically, the reaction mixture contains an acetylcholine-like substance. (4) Myosin *B*[4] extracted from skeletal, heart and plain muscle acts like acetylcholinesterase (recently, similar results have been reported by Hungarian workers[5]).

Two suggestions arise from these results and several other facts, namely, (1) the glycerinated muscle fibres are considered to have no active plasma membrane[4,6]; (2) the present experiments on the fibres were made under the condition of 0·16 *M* potassium chloride (in other words, potassium-paralysis); (3) if the fibres are fresh after the preparation, they have some systems other than actomyosin-system, for example, the relaxing factor[7]; (4) sarcoplasmic reticulum is supposed to have an electrically polarized membrane like plasma membrane[8,9]. One suggestion is the possibility that in general not only in muscle cells but also in other excitable cells, such as nerve, the intracellular granules (or endoplasmic reticulum) may liberate acetylcholine and/or the other active substances such as adrenaline by electrical stimulation, suggesting some intracellular roles of action current. The other is related to the mechanism by which the excitation of plasma membrane of a muscle fibre evokes the contraction of myofibrils, that is, the linking mechanism in question could be easily explained as in the case of the ordinary synapse. If this mechanism is accepted, the new 'synapse' linking the excitation of the plasma membrane with the events within the cell should be called an 'inner synapse'.

Since there are some objections[10], however, to the view postulating the direct role of the action current with respect to the linking mechanism, great caution

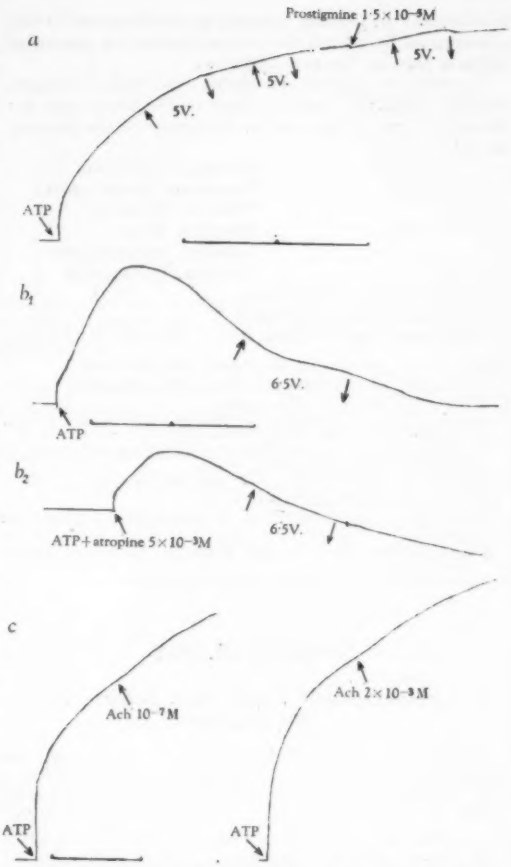

Fig. 2. Influence of prostigmine (*a*) and of atropine (b_1 and b_2; b_1 is control) on the enhancement of the adenosine triphosphate-contraction of the glycerinated fibre bundle by electrical stimulation, and effect of acetylcholine on the adenosine triphosphate-contraction of the bundle (*c*). Signs and conditions are similar to the case in Fig. 1. Atropine was contained in the solution with adenosine triphosphate. Prostigmine and acetylcholine were added at the point indicated by arrows

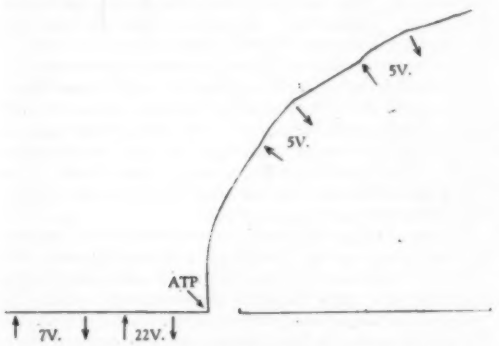

Fig. 1. Influence of electrical stimulation on the isotonic adenosine triphosphate-contraction of glycerinated muscle fibres. Time interval is 1 min. At adenosine triphosphate ↓ the bathing fluid of 0·16 *M* potassium chloride was exchanged with a new fluid of 10 ml. consisting of 0·16 *M* potassium chloride, 4 m*M* adenosine triphosphate, 4 m*M* magnesium chloride and 20 m*M* *tris*-hydroxymethyl-aminomethane (*p*H 7). A train of repeated induced current generated by Porter-type induction coil was applied between the two signs, ↑ and ↓, through two platinum-electrodes, which were placed about 8 cm. apart at the ends of the bathing fluid. Applied voltages between the two electrodes are given in the figure. Temperature (23° C.) was regulated with water surrounding the reaction tube connected with a thermostat, and the bathing fluid immersing the fibre bundle was stirred by air-bubbling. The fibre bundle used was about 30 mm. × 0·3 mm. of 7 days after preparation

is necessary in putting forward an explanation of the mechanism by which the action current or potential plays a part in the new synapse.

We wish to express our thanks to Prof. T. Nagai, Prof. E. Miyazaki and members of his laboratory for their kind encouragement in the course of the present study.

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[1] Kahn, A. J., and Sandow, A., *Science*, **112**, 647 (1950).
[2] Bay, Z., and Szent-Györgyi, A., *Nature*, **167**, 482 (1951).
[3] Csapo, A., *Nature*, **173**, 1019 (1954).
[4] Szent-Györgyi, A., "Chemistry of Muscular Contraction", 2nd ed. (Academic Press, New York, 1951).
[5] Kovács, T., Kövér, A., and Szöör, A., Fourth Internat. Congr. of Biochem., Vienna, 83 (1958).
[6] Fenn, W. O., *Proc. Soc. Exp. Biol. Med.*, **96**, 783 (1957).
[7] Marsh, B. B., *Nature*, **167**, 1065 (1951).
[8] Porter, K. R., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **3**, 269 (1957).
[9] Ruska, H., Edwards, G. A., and Caesar, R., *Experientia*, **14**, 117 (1958).
[10] Sten-Knudsen, O., *J. Physiol.*, **125**, 396 (1954).

HÆMATOLOGY

Haptoglobin Types in a Japanese Population

Since the discovery by Smithies[1] that there are genetically controlled types in human haptoglobins, a few reports[2–5] on the distribution of these types in different populations have been published. These are, so far as we are aware, concerned with Caucasian and Negro peoples, with the one exception—that by Sutton *et al.*[5] on a certain tribe of American Indians. In view of both anthropological and genetical interest of such population studies, some results of our investigations will be reported here.

Employing Smithies's method[1], starch gel electrophoresis was performed on the sera of 349 unrelated adults which were selected at random. They were all apparently healthy and living in Sapporo. Oxyhæmoglobin was added to a concentration of about 100 mgm. per 100 ml. prior to electrophoresis. The resultant starch strips were treated with a benzidine reagent and the haptoglobin types were read immediately. Amido black 10*B* staining was then carried out. Moreover, in some of the sera an estimate was made of the amount of serum haptoglobins in terms of their hæmoglobin binding capacity by the method of paper electrophoresis described by Nyman[6], using phosphate buffer of *p*H 7·0 and a leucomalachite green reagent for the detection of hæmoglobin–haptoglobin complexes. This was especially useful for confirmation of the diagnosis of type 1–1 and ahaptoglobinæmia, since the apparent similarities of the patterns of these two may occasionally lead to erroneous classification if the method of starch gel electrophoresis only is employed.

The frequencies of the types 1–1, 2–1 and 2–2 among the 349 persons were 21, 122 and 201, respectively. No samples with modified 2–1 type[4,7,8] have so far been encountered. The 201 of type 2–2 include one person in whom, though at the first examination no haptoglobin could be demonstrated, at the second examination performed one month later, the type 2–2 pattern was slightly but unmistakably present. In the remaining five individuals it was impossible to demonstrate haptoglobins by repeated examinations.

If one ignores the five ahaptoglobinæmics, the test for genetic equilibrium of the frequencies of three haptoglobin types shows a good agreement of our observation with the genetic hypothesis by Smithies and Walker[9] ($\chi^2 = 0{\cdot}186$, $d.f. = 1$, $0{\cdot}70 > P > 0{\cdot}50$). Based on the above results, the frequency of the gene Hp^1 for the Japanese population may be estimated as 0·238 with standard error of 0·016. Comparing this value with those obtained by Sutton *et al.*[5] for other populations, the frequency of Hp^1 among Japanese is apparently lower than in Caucasians and Negroes, but slightly higher than that in Asiatic Indians.

The incidence of ahaptoglobinæmics in our results, five or 1·4 per cent of the 349, is apparently lower than that reported for Nigerians[3] or American Negroes[4,5], but perhaps higher than that in Caucasians[10,11]. This may suggest that such racial differences do exist, as pointed out by Allison *et al.*[3,11], even though the stated incidences may include not only primary but also secondary ahaptoglobinæmics due to certain environmental causes. Four out of the five had no history of anæmia or liver disease. The fifth, a 37-year old woman, had a history of hepatitis seven years ago, but neither clinical examination nor laboratory tests revealed any hypofunction of the liver or elsewhere at present. Follow-up examinations, as well as family studies, of these ahaptoglobinæmics are now in progress.

In order to compare the amount of haptoglobins between the three types, the hæmoglobin binding capacity value was determined in the sera from 67 'healthy' women aged 19–30. The mean values (mgm. per 100 ml.) were found to be $131{\cdot}3 \pm 14{\cdot}9$ in type 1–1, $121{\cdot}2 \pm 8{\cdot}1$ in type 2–1 and $95{\cdot}5 \pm 6{\cdot}7$ in type 2–2. The results agree with those reported by Nyman[6,10], indicating that the haptoglobin synthesized by the gene Hp^1 is either more in amount or has higher capacity of binding hæmoglobin than that produced by the gene Hp^2. Sutton *et al.*[5] found that in about half the 74 Asiatic Indians examined, the haptoglobin concentrations were unusually low and they noted a racial variation in the haptoglobin-levels. From our results it seems that there is probably no difference in this respect between Japanese and Caucasians.

In connexion with the occurrence of ahaptoglobinæmics among 'healthy' individuals, sera from patients admitted in the Sapporo Medical College Hospital are now being examined, on whom the diagnosis of the diseases seem to have been established. Ahaptoglobinæmia has so far been encountered in all four patients with liver cirrhosis examined, in the single case of schistosomiasis japonica and in another one of chronic endocarditis lenta studied. Of the six patients with gastric carcinoma examined, no haptoglobin has been demonstrated in one in whom metastasis to the liver had occurred. In one of the three patients with duodenal ulcer examined, and in one of the three with hepatoma, the haptoglobins were scarcely recognized. Pathological implications of these findings will be the subject of further investigation.

It is a pleasure to acknowledge the hospitality of the Department of Human Genetics, University of Michigan, particularly Drs. J. V. Neel and H. E. Sutton, during May 1958, who gave one of us (E. M.) the opportunity of learning the technique of starch-gel electrophoresis. Thanks are also due to Dr. T. Wada of the Department of Internal Medicine, Sapporo Medical College, who kindly helped us in obtaining pathological sera.

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[1] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[2] Sutton, H. E., Neel, J. V., Binson, G., and Zuelzer, W. W., *Nature*, **178**, 1287 (1956).
[3] Allison, A. C., Blumberg, B. S., and ap Rees, W., *Nature*, **181**, 824 (1958).
[4] Giblett, E. R., *Nature*, **183**, 192 (1958).
[5] Sutton, H. E., Neel, J. V., Livingstone, F. B., Binson, G., Kunstadter, P., and Trombley, L. E., *Ann. Human Genet.*, **23**, 175 (1959).
[6] Nyman, M., *Clin. Chim. Acta*, **3**, 111 (1958).
[7] Connel, G. E., and Smithies, O., *Biochem. J.*, **72**, 115 (1959).
[8] Galatius-Jensen, F., *Acta Genet.*, **8**, 248 (1958).
[9] Smithies, O., and Walker, N. F., *Nature*, **178**, 694 (1956).
[10] Nyman, M., "Serum Haptoglobin" (Lund, 1959).
[11] Allison, A. C., *Nature*, **183**, 1312 (1959).

Agar-gel Electrophoretic Demonstration of Individual Variations in Electrophoretic Position of an Indoxyl-acetate Splitting Component in the α_2 Globulin Region of Normal Human Sera

In a previous communication[1], qualitative differences in immuno-electrophoretic pattern of human sera were demonstrated, which were found to be genetically controlled[2]. On account of a fast-moving precipitating component (group 1), a precipitate with two peaks (group 2) and a slowly moving precipitating component (group 3), all sera hitherto investigated could be grouped into any of these types, including a few sera which were found to lack any haptoglobin as demonstrated by starch-gel and agar-gel electrophoresis[1,3]. These groups were further found not to be identical with the haptoglobin groups[1,3].

In order to study their possible relation to the three different cholinesterase types, recently demonstrated by Kalow, by means of inhibitor experiments[4], agar-gel electrophoresis[3] was performed on 12 normal sera. The agar-gel electrophoretic pattern was thereafter developed with an indoxyl–acetate reagent (Heilbronn, E., personal communication). By this technique, differences in electrophoretic position of an indoxyl-acetate splitting component in the α_2-region was detected. These 12 sera could therefore be grouped into several types, which were preliminarily characterized as fast, intermediate and slow. In order to study the reproducibility of the technique, two sera showing the fast-moving component and two sera showing the slowly-moving component were subjected to renewed agar-gel electrophoresis on two different slides. These differences were thereby found to be reproducible.

By means of agar-gel electrophoresis[3], the haptoglobin groups of these 12 sera were determined by means of benzidine staining after addition of hæmoglobin to the sera. These experiments were performed in duplicates on two 8 cm. × 8 cm. slides and showed absolute correlation in electrophoretic position as determined by the naked eye.

Table 1

Serum No.	Haptoglobin group	Immuno-electrophoretic group	Position of indoxyl–acetate-splitting component
5/173	2–2	1	slow
1/80	1–1	3	intermediate
5/170	1–1	1	intermediate
5/97	2–1	3	intermediate
3/169	2–2	1	intermediate
4/97	2–1	2	intermediate
1/174	2–1	2	fast
7/182	2–1	1	intermediate
13/178	2–1	1	fast
4/182	2–2	2	slow
2	2–2	1	intermediate
4	1–1	3	—

Relation of indoxyl-acetate-splitting component in twelve different human sera as compared to their haptoglobin and immuno-electrophoretic groups.

The immuno-electrophoretic groups were determined by means of immuno-electrophoresis, according to previously published techniques[1,5]. The results are given in Table 1. In this table are also included two reference sera (2 and 4) which were kept in the deep-freeze for one year and have been repeatedly thawed and investigated, without showing any change of immuno-electrophoretic groups. No indoxyl-stainable component could be detected in one of these sera, and it was very weak in the other, thus suggesting that these components are susceptible to storing.

The other sera included in Table 1 represent normal sera from adult women (with the exception of 5/97, which is a serum from an infant) which were kindly given by Prof. Birger Broman, State Institute for Blood Group Serology, Stockholm, and have all been stored in the deep-freeze for about two months.

Samples from four blood donors (not included in Table 1) showed that the groups were constant for at least one year in these blood donors—three of which were found to belong to the intermediate type and one to the slow-moving type.

These investigations are continuing, and the genetical significance as well as possible relation to the cholinesterase types demonstrated by means of inhibitor-experiments[4,6] will be studied.

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[1] Hirschfeld, J., *Acta Path. et Microbiol. Scand.*, **47**, 160 (1959).
[2] Hirschfeld, J., *Nature* (in the press).
[3] Hirschfeld, J., *Acta Path. et Microbiol. Scand.*, **47**, 169 (1959).
[4] Kalow, W., and Staron, N., *Can. J. Biochem. Physiol.*, **35**, 1305 (1957).
[5] Hirschfeld, J., *Acta Path. et Microbiol. Scand.*, **46**, 229 (1959).
[6] Harris, H., and Whittaker, M., *Nature*, **183**, 1808 (1959).

Blood Groups in Rabbits

The blood groups of rabbits were first reported in 1929[1] and since then many letters have been suggested to designate them[2–9]. Recently, attempts have been made to compare the various nomenclatures of the different investigators[10].

Experiments in our laboratory were conducted in order to investigate the rabbits' red cell antigens in connexion with tissue homografting experiments. The nomenclature used has been suggested by Cohen[10]. Our A (genotypes Hg^A/Hg^A and Hg^A/Hg^D) and F (genotypes Hg^F/Hg^F and Hg^D/Hg^F) blood

types corresponded to the A and B antigens of Anderson[8], who kindly provided us with antisera.

In our experiments 250 local rabbits were examined. The blood group of each rabbit was determined by adding one drop of a 20 per cent red blood cell suspension to 0·1 ml. of antiserum in a small test-tube, incubating for 1 hr. in a 37° C. water-bath, and examining for macroscopical agglutination. Table 1 summarizes the results of these determinations.

Table 1. Blood Group Distribution in 250 Rabbits

Total	A	F	D	AF
250	64	88	30	68
100	24	33	19	24 per cent

100 rabbits were examined for the presence of natural anti-A or anti-F antibodies in their sera. In 97 rabbits no *iso*agglutinins were found, while the undiluted serum of only 3 rabbits agglutinated erythrocytes of the corresponding group.

Immunization experiments were conducted in order to produce anti-A and anti-F sera. No adjuvant was used in any of the experiments. 2-kgm. rabbits were injected with thrice washed red blood cells of the corresponding blood group, as follows: 10 ml. of a 20 per cent erythrocyte suspension in physiological salt solution was injected in each case into different sites. The first immunization was carried out intravenously, intracutaneously into the footpads and intramuscularly. In the following immunizations the intravenous route was omitted. The immunizations were repeated twice weekly for 7 weeks. At this time about half the rabbits produced a good titre and blood was withdrawn for test serum. The titre was about 1 : 128 in saline, and 1 : 1,024 in 6 per cent dextran, or with papain-treated erythrocytes. Before use the serum was inactivated, and diluted to have a titre of 1 : 32 in saline. The incomplete (dextran, papain) antibodies were found to appear earlier during the immunization and attain a higher titre than the complete antibodies.

In the course of two immunization experiments for preparation of anti-A and anti-F sera, it has been observed that the sera behaved in an unexpected way. The anti-A serum agglutinated apart from all A and AF cells also some D and F erythrocytes, and the anti-F serum agglutinated A and D erythrocytes. These findings suggested the presence of an additional antibody in these sera capable of agglutinating about 70 per cent of the erythrocytes of all rabbits. With the aid of these sera D rabbits were divided into two groups: one reacting with the sera, having the additional antigen, which we designated D(X+), and the second not reacting with the sera and therefore lacking the antigen, which we designated D(X−).

These rabbits were arranged in pairs, so that D(X−) rabbits were immunized with D(X+) erythrocytes. Later, F(X−) and AF(X−) rabbits were also immunized in a similar way, the same donor always immunizing the same recipients. All the X-negative rabbits produced antibodies against the X-positive blood cells active only in dextran or with papain-treated cells. The titres obtained were found not to be higher than 1 : 128 and no saline agglutinins were detected in any of the experiments.

As controls F(X+) and AF(X+) rabbits were immunized with F(X−) and D(X−) erythrocytes respectively. None of these rabbits produced any antibodies.

With the aid of the new anti-X serum 100 random rabbits were examined of which 75 were found to be X-positive and 25 X-negative. Table 2 summarizes the results of these experiments.

Table 2. Distribution of the X-Antigen in 100 Rabbits in Relation to the A, F, D Groups

A		F		D		AF	
+	−	+	−	+	−	+	−
15	1	19	17	11	3	30	4

These findings suggest another antigen system of the rabbits' red blood cells. The blood factor X is not identical with either the antigen J or I reported by Cohen[10] as the X factor is not limited to the Hg^A/Hg^F or Hg^A/Ag^D groups but has been found also in the Hg^D/Hg^D(D) group. It is felt that the growing interest in the rabbits' antigens makes the co-operation and exchange of antisera between the different workers most desirable.

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[1] Levine, P., and Landsteiner, K., *J. Immunol.*, **17**, 559 (1929).
[2] Castle, W. E., and Keeler, C. E., *Proc. U.S. Nat. Acad. Sci.*, **19**, 92 (1933).
[3] Fisher, W., *Z. Imm. Forsch.*, **86**, 97 (1935).
[4] Marcussen, P. V., *Z. Imm. Forsch.*, **98**, 453 (1936).
[5] Heard, D. H., Hinde, I. T., and Mynors, L. S., *J. Hyg.*, **49**, 119 (1949).
[6] Kellner, A., and Hedal, E. F., *J. Exp. Med.*, **97**, 33 (1953).
[7] Joysey, V. C., *J. Exp. Biol.*, **32**, 440 (1955).
[8] Dahr, F., Fischer, K., and Kindler, M., *Z. Hyg.*, **141**, 91 (1955).
[9] Anderson, J. R., *Brit. J. Haemat.*, **1**, 378 (1955).
[10] Cohen, C., *Transpl. Bull.*, **5**, 21 (1958).

PATHOLOGY

Graft Versus Host Reaction in Parabiotic Disease

In an attempt to induce tolerance to skin homografts in young and adult albino rats the technique of pedicled grafting combined with parabiosis was used. 26·2 per cent of recipients thus challenged with a homologous skin transplant failed to destroy the graft and died of an illness which will be referred to as 'parabiotic disease'[1]. So far, all hosts unable to destroy the graft and weighing less than 160 gm. died of this disease. Parabionts weighing more than 160 gm. may show tolerance to homografts without manifest signs of the disease (unpublished results).

The initial symptom of parabiotic disease is diarrhœa, which is soon followed by acute hæmolytic anæmia and the animal may die in a few hours. In less acute cases the animal fails to grow or suddenly loses weight, becomes anæmic and emaciated, breathes heavily, nose and mouth are covered with bloody discharge, coat ruffled, skin atrophic. The atrophy of lymph glands is a constant finding.

It was suggested[1] that parabiotic disease was caused by an immune reaction of grafted cells against their host. Two possible sources of antibody formation were mentioned: (*a*) the skin graft itself; (*b*) the blood cells exchanged between parabionts after establishment of vascular anastomoses. The purpose of the present experiment was to show the role of these two sources of antibody formation in the causation of parabiotic disease.

Ninety albino rats of both sexes, weighing 90 to 150 gm. from two unrelated random-bred strains, *Y* and *W*, were used. Partners never belonged to the same strain and were of the same weight and sex. The animals were divided into two groups. Group I consisted of 50 animals which were joined in parabiotic union (25 pairs) without receiving any grafts; group II consisted of 40 animals which were joined in parabiotic union (20 pairs) and free, full thickness skin grafts were exchanged between the partners. Parabionts of both groups were separated on the fifth post-operative day.

In group I four animals (8 per cent) died of parabiotic disease; three of them died on the sixteenth and one on the seventeenth post-operative day. In group II, two parabionts (5 per cent) carrying preserved grafts died of parabiotic disease on the sixteenth and the eighteenth post-operative day respectively. The rest, that is, thirty-eight parabionts, destroyed their grafts without manifest signs of the disease. Survival times of grafts are shown in Table 1 as are also normal median survival times of grafts transplanted from *W* to *Y* rats and vice versa[1].

Table 1. SURVIVAL TIME OF FREE SKIN GRAFTS EXCHANGED BETWEEN PARABIONTS

Strain combination	Survival times of skin grafts (days)	Median survival time (days)*
$W \rightarrow Y$	8, 8, 8, 8, 8, 8, 8, 9, 9, 9, 9, 9, 10, 11, 11, 12, 14, 15, 19	$10{\cdot}2 \pm 0{\cdot}7$
$Y \rightarrow W$	8, 9, 9, 9, 9, 9, 9, 11, 11, 11, 11, 11, 12, 14, 15, 15, 16, 20, 20	$12{\cdot}1 \pm 0{\cdot}8$

* Normal median survival time of skin grafts transplanted from *W* to *Y* rats and vice versa are as follows: $W \rightarrow Y$, $8{\cdot}1 \pm 0{\cdot}4$ days; $Y \rightarrow W$, $10{\cdot}2 \pm 0{\cdot}4$ days. No graft survives beyond the twelfth day (ref. 1).

Since only one partner in a parabiotic pair may die of parabiotic disease the other partner cannot be included in any computation showing mortality-rate. Therefore, the term 'halved pair' should be used for this purpose. So 16 per cent of halved pairs in group I and 10 per cent of halved pairs in group II died of parabiotic disease.

As can be seen from the results, parabiotic disease can manifest itself in separated parabionts which have received no skin grafts (group I). The presence of a free skin graft (group II) does not increase the incidence of parabiotic disease but rather the reverse. A free skin graft seems to be a poor producer of antibodies, which is not surprising if we remember that a normal animal challenged with a skin graft will destroy it without ever showing any signs of graft versus host reaction even when immune reaction has been postponed (X-rays, cortisone, etc.). Graft versus host reaction is seen only in those cases where a recipient has been permanently or temporarily deprived of its immune potential and challenged with homologous cells capable of reacting against their host. Unborn or newly born mice injected with spleen cells from an adult homologous donor develop 'runt disease'[2]. A similar condition has been described in chicks[3]. Lethally irradiated adult mice treated with homologous spleen and marrow cells die of 'secondary disease'[4]. Obviously, the source of immune attack in our cases does not rest in a free skin graft but must be sought elsewhere. We think we are justified in supposing that adult blood cells exchanged between parabionts may survive in an adult host and react immunologically against it. The case might be somewhat different when a parabiont is challenged with a pedicled skin graft[1]. The difference in mortality-rate between parabionts with a pedicled graft and parabionts in the present series might be explained if we supposed that a pedicled graft increased the number of immunologically active cells. During parabiotic union reticulo-endothelial cells from the donor could migrate through the pedicle into the graft and thus condition it against the host. Other factors such as humoral might also play an important part. The incidence of parabiotic disease in permanent parabiosis is much higher (around 70 per cent)[5] because the parabiotic partner acts as an immunologically active graft.

The fact that free, full thickness skin grafts survive longer if exchanged between parabionts than between normal animals (Table 1) is not easily explained and might be due to various reasons, such as trauma, immunoparalysis, enhancement, etc.

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[1] Nakić, B., and Silobrčić, V., *Nature*, **182**, 264 (1958).
[2] Billingham, R. E., and Brent, L., *Transpl. Bull.*, **4**, 67 (1957).
[3] Simonsen, M., *Acta Path. Microbiol. Scand.*, **40**, 480 (1957).
[4] Ilbery, P. L. T., Koller, P. C., and Loutit, J. F., *J. Nat. Cancer Inst.*, **20**, 1051 (1958).
[5] Finerty, J. C., *Physiol. Rev.*, **32**, 277 (1952).

Effect of Molecular Structure upon Concentration of Hydrocarbons in 'Painted' Mouse Skin

ALTHOUGH several hypotheses have related molecular structure to carcinogenicity of polynuclear hydrocarbons[1], little has been done to correlate structure with specific steps in the overall process. We have studied one important step, the uptake and retention of hydrocarbons by exposed tissue.

Strong *A* mice were used at an age when the hair was resting and the skin was uniformly thin. 0·25 ml. of a 1 per cent solution of hydrocarbon in 99 : 1 benzene-mineral oil was applied to the clipped back of each animal. After 120 min., the mice were killed, hair stubble was removed with barium sulphide, and the intact skin was scrubbed repeatedly with fresh benzene. Treated skin from each animal was weighed, minced, and heated at 60° C. for about 30 min. with 1·0 ml. of 95 per cent ethanol and 1·0 ml. of 10 per cent aqueous potassium hydroxide. The homogenate then was thoroughly mixed with 5·0 ml. of benzene and 5·0 ml. of water. The benzene layer was obtained and, when necessary, anhydrous sodium sulphate was added to remove traces of water still in suspension. The individual benzene extracts were analysed in an Aminco-Bowman spectrophotofluorometer.

The results are presented in Table 1. Concentration being independent of sex, separate values are not given. An important factor affecting localization within the skin is molecular dimensions. Thus phenanthrene-levels were much greater than those of other hydrocarbons. Likewise, compounds with methyl substituents generally gave lower values than did the parent ring compounds. However, size is not

Table 1. Concentration of Hydrocarbons in Mouse Skin after 'Painting'

Compound	No. of mice	Concentration in skin after 120 min. (μgm./gm. ± S.E.)	Carcinogenic potency (ref. 2)
6-Methylbenzo(a)-pyrene	8	5·22 ± 0·51	+++
7-Methylbenz(a)-anthracene	8	5·48 ± 0·84	++
3-Methylcholanthrene	36	8·85 ± 0·65	+++
Benz(a)anthracene	14	9·76 ± 1·16	±
Benzo(a)pyrene	16	11·47 ± 1·06	+++
Benz(a)acridine	8	12·52 ± 1·26	−
7,9-Dimethylbenz(c)-acridine	16	15·18 ± 1·04	+++
Anthracene	8	16·95 ± 2·34	−
7-n-Propylbenz(a)-anthracene	8	18·06 ± 1·99	−
Benz(c)acridine	8	22·32 ± 1·85	−
7,12-Dimethylbenz(a)-anthracene	8	23·12 ± 1·32	+++
Phenanthrene	8	93·5 ± 19·6	−

the sole property affecting localization. There are marked differences between benz(*a*)anthracene and the two benzacridines. Further, in the case of dimethylbenz(*a*)anthracene and propylbenz(*a*)anthracene, the presence of the alkyl groups caused increased concentrations. In general, high *K* region activity[1] appears to favour increased concentration of the compound within the skin. There is no direct relationship between carcinogenic potency and skin concentrations.

Tissue concentrations depend upon rates of penetration, metabolism, and removal of compounds from the skin. Current studies are being undertaken to differentiate among these mechanisms.

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[1] Pullman, A., and Pullman, B., "Advances in Cancer Research", edit. by Greenstein and Haddow, **3**, 177 (Academic Press, Inc., New York, 1955).

[2] Hartwell, J. L., "Survey of Compounds which have been Tested for Carcinogenic Activity", 2nd ed. (U.S. Gov. Printing Office, Washington, D.C., 1951). Shubik, P., and Hartwell, J. L., "Survey of Compounds which have been Tested for Carcinogenic Activity", Supp. 1 (U.S. Gov. Printing Office, Washington, D.C., 1957).

Combined Application of Hydrogen Peroxide and Ionizing Rays in Animal Experiments

Holman[1] has reported the effect of 0·45 per cent hydrogen peroxide as drinking water on rats injected with Walker carcinosarcoma. He found that within a period of sixty days approximately 50–60 per cent of the animals were cured. Of seventy-two animals, he observed ten for a period of more than two months. Further, he reported the use of hydrogen peroxide in the treatment of four patients with advanced inoperable tumours, two of whom showed an improvement.

Makino[2] investigated the effect of injecting hydrogen peroxide intraperitoneally into rats which had been inoculated six days previously with Yoshida ascites sarcoma. 2 hr. after an injection of 1 ml. of 2 per cent hydrogen peroxide he found 90 per cent of the tumour cells to be injured; also he found similar damage in the neutro- and eosino-philic leucocytes. Some of the tumour cells were completely uninfluenced by this hydrogen peroxide.

Holman's methods of procedure were checked by Ghadially[3], Koloczek[4] and Sugiura[5], who injected rats with Walker carcinoma 256 and also injected mice and rats with other tumours. These authors could not, however, confirm Holman's results.

First of all, we tried to cure rats with Walker carcinosarcoma 256 following Holman's method, but we could not observe a cure. Therefore we altered his method in order to obtain eventual tumour inhibition.

A total of 516 rats were tested in eight separate experiments, each with four sub-groups of from 10–30 animals. The rats are descended from our own inbreeding of a Wistar strain; the uniform feeding and tending of them are guaranteed. The rats in a single experiment were injected at the same time subcutaneously with Walker carcinosarcoma. After several days the tumours were palpated and therapy was begun on those rats with a tumour weight of 1–2 gm. The experimental groups were arranged symmetrically, large tumours and small tumours distributed equally. In the separate experiments the animals were divided into four groups as follows.

Group	Therapy
A	Hydrogen peroxide
B	X-rays
C	Hydrogen peroxide and X-rays
D	Untreated controls

The animals of group *A* received 0·45 per cent hydrogen peroxide solution as drinking water *ad lib.*

The peroxide solution was freshly prepared daily. With the animals of group *B* X-irradiation was employed over a period of four days. Radiation was given by a Philips contact X-ray apparatus (2 m.amp. 1 mm. aluminium filter, corresponding to a half-value layer of 1·12 mm. aluminium, with a 4·5-cm. tube; there was exact draping of the healthy regions. The total dose entering the tumour base amounted to 3,000 r.

Group *C* received X-rays, and hydrogen peroxide as drinking water.

Group *D* remained completely untreated as controls.

Hydrogen peroxide was given in general until the death of the experimental animals.

The rats were killed 7–10 days (in one experiment 4 days) after the beginning of the therapy, after which the tumours were prepared, the weight of the experimental animals determined, the weight of the free prepared tumours recorded, the volume of the tumours measured by water displacement, and the degree of the subcutaneous necrosis judged.

The results of the eight separate experiments were essentially the same. Hydrogen peroxide alone decreased the body-weight of the animals to an extent of 10 per cent compared to the untreated control group. This effect did not appear in all experimental groups.

The tumour-weight of the group treated only with hydrogen peroxide showed a small, but not significant, difference as compared with the untreated control group.

The dose of X-irradiation showed a clear inhibitory effect on tumour growth. The combination of X-rays and hydrogen peroxide, however, did not increase this inhibitory effect. In one case a small increase in the inhibitory influence was noticeable; but this difference was by no means significant.

In the untreated control group there was in every instance a strong increase in weight of the subcut-

aneous tumours without the appearance of noticeable spontaneous regressions.

The small tumour inhibition could, in our opinion, be explained by reduction in the body-weight of the animals, which we could also observe in those animals which had hydrogen peroxide in the drinking water; Ghadially and Koloczek have already referred to this.

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[1] Holman, R. A., *Nature*, **179**, 1033 (1957).
[2] Makino, S., and Tamaka, T., *Gann*, **44**, 39 (1953).
[3] Ghadially, F. N., and Wiseman, G., *Nature*, **181**, 1067 (1958).
[4] Koloczek, B., Molawka, E., Penar, B., and Szweda, Z., *Nature*, **182**, 395 (1958).
[5] Sugiura, Kanematsu, *Nature*, **182**, 1310 (1958).

PLANT PHYSIOLOGY

An Enzymic Basis for Pathogenic Specificity

A substance present in oat roots and leaves which inhibits growth and respiration of several fungi has been described[1]. It was suggested that the resistance of oats to *Ophiobolus graminis*, the cause of take-all of wheat, and its susceptibility to the very similar *O. graminis* var. *avenae*, was connected with this inhibitor, since var. *avenae* (*O* isolates) grew in sap expressed from oat roots whereas *O. graminis* itself (*W* isolates) did not. However, crudely purified inhibitor seemed equally toxic to both. This inhibitor has now been purified by H. K. Mitchell and J. V. Maizel and named 'avenacin' (personal communication). They find it to be identical with a fluorescent glucoside described as occurring in oat root meristems by Goodwin[2]. They give the ED_{50} against *Neurospora crassa* growing in Fries medium as 0·4 μgm./ml. They find that on hydrolysis in 0·1 *N* hydrochloric acid, it loses its biological activity, and glucose and an unidentified pentose are released (Maizel, J. V., and Mitchell, H. K., unpublished work).

I have examined the culture filtrates of both varieties of *O. graminis* and of some other fungi for glucosidase activity against avenacin and have found activity only in *O. graminis* var. *avenae* (isolates O_2 and O_8). The method described by Jermyn[3] for obtaining concentrated samples of β-glucosidase from culture filtrates was used. This provided 10 ml. of enzyme extract from 100 ml. of culture filtrate. To this was added 50 μgm. of avenacin (0·5 ml. of an 80 per cent ethanol solution containing 100 μgm./ml.). After incubating at 33° C. for 24 hr., it was evaporated to dryness, taken up in 1·5 ml. ethanol, filtered, and 0·5 ml. applied to a single spot on Whatman 3 *MM* paper. The chromatogram was run (ascending) in propanol/1 per cent ammonia (3 : 1) and then examined under ultra-violet light. Fig. 1 shows that the R_F value of avenacin is 0·72, and that of the fluorescent aglycon obtained after hydrolysis with 0·1 *N* hydrochloric acid or with the extracted enzyme from culture filtrates of O_2 and O_8 is 0·91. The other fungi examined have no activity. Boiling the preparation from O_2 destroys activity. A sample of commercial β-glucosidase is also inactive; α- and β-glucosidases

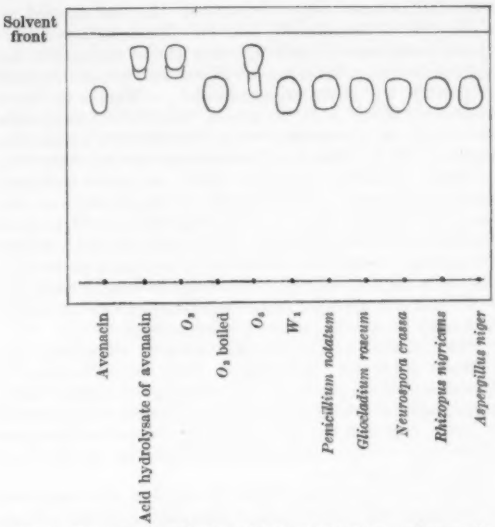

Fig. 1. Chromatogram showing the effect of treatment of avenacin with extracts from culture filtrates of various fungi. Spots visible under ultra-violet lamp

are known to be of widespread occurrence in fungal culture media and Jermyn found high β-glucosidase activity for *Penicillium notatum* and *Gliocladium roseum*, both inactive here. Both *W* and *O* culture filtrates showed α-glucosidase activity against maltose. The enzyme concerned in hydrolysis of avenacin therefore seems to be specific. Loss of inhibitory power by avenacin on enzymic hydrolysis was confirmed by growth experiments (Table 1).

Table 1. Growth of *Neurospora crassa* after 18 hr. on 0·1-ml. streaks of agar containing 0·05 ml. of avenacin solution recovered from enzyme extracts from W_1 and O_8

Additions to nutrient agar	Average diameter of four colonies
Nil	25 mm.
W_1 preparation	14 mm.
O_8 preparation	22 mm.

Glucosidase activity was lost after 24 hr. and was much reduced by adding 2 drops of hydrogen peroxide solution to the enzyme extract. The other fungi tested, with the exception of *Neurospora*, are all insensitive to avenacin, but clearly not because they produce an inactivating enzyme.

The production of an enzyme inactivating an inhibitor in a potential host plant can be compared to a mutation to production of penicillinase by soil bacteria such as *B. cereus*. In the present case, oats can be regarded as having a built-in 'antibiotic', avenacin, which protects it against infection by *O. graminis*. A mutation to production of the specific glucosidase would enable the pathogen to increase its host-range. There is no resistance to penetration of oat roots by *O. graminis*, which ramifies in the cortical cells of the seedling roots but then dies and the infection is thrown off. A mutation in any of the hyphal cells to production of the enzyme would enable the infection to develop. Oats is frequently included in the rotation between crops of wheat in order to control take-all, and transitory invasion of the seedling roots by *O. graminis* may frequently occur.

A mutation to glucosidase production, however, would not by itself account for the existence of var. *avenae*, which is distinguishable from *O. graminis* by two other characteristics: (*a*) mean length ascospore

(recorded means range from 96 to 123μ compared to 70–90μ for *O. graminis*[4]), and (*b*) all 8 isolates of var. *avenae* examined physiologically show inhibition by cystine and cysteine whereas *W* isolates are stimulated in growth by these amino-acids[5]. There is some evidence (which will be given elsewhere) that this sensitivity is connected with the greater reductive capacity of *O* isolates. Glucosidases are inhibited by oxidizing agents[6] and for the avenacin-splitting enzyme to act *in vivo* it may be necessary for the mycelium producing it to maintain the environment in a low state of oxidation. A mutation to glucosidase production would then be effective only in a mycelium with this high reducing power. Spore-length may not be a valid criterion for distinguishing the two varieties[4] or it may be a subsequent development.

The inhibitor/inactivating-enzyme complex described here as accounting for the pathogenic specificity of *O. graminis* is probably too crude to be concerned in the extremely precise specificity found in rusts, but it may play a part in other cases where inhibitory conditions in certain varieties of the host seem to be involved.

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[1] Turner, E. M., *J. Exp. Bot.*, **7**, 80 (1956).
[2] Goodwin, R. H., and Kavanagh, F., *Bull. Torrey Bot. Club*, **75**, 1 (1948).
[3] Jermyn, M. A., *Aust. J. Biol. Sci.*, **12**, 213 (1959).
[4] Garrett, S. D., and Dennis, R. W. G., *Trans. Brit. Mycol. Soc.*, **26**, 146 (1943).
[5] Turner, E. M., *J. Gen. Microbiol.*, **16**, 531 (1957).
[6] Hestrin, S., Feingold, D. S., and Schramm, M., "Methods in Enzymology", **1**, 240 (1955).

An Ion-Exchange Resin Method for the Fractionation of Alcoholic Plant Extracts

During a study of the effect of benzimidazole on the carbon-14 labelled amino-acid metabolism in detached wheat leaves, it was found desirable to fractionate an alcoholic extract (less the chloroform-soluble substances) into acidic, basic, and non-ionic (or neutral) substances prior to any further chromatographic analysis on filter paper. Ion-exchange resins offer a valuable means for this purpose, which may be satisfactorily achieved with a combination of cationic and anionic ion-exchangers. I have, however, experienced a considerable loss of radioactivity with such ion-exchangers as 'Amberlite *IR*-120' and '*IR*-45' and, as a result, recovery of the radioactive compounds was never quantitative. Later it was found that a combination of 'Dowex 50, *X*-8' (200–400 mesh) and '*AG* 1 (Dowex 1)', '*X*-10' (200–400 mesh) in formate form made it possible to fractionate the alcoholic extract into basic, acidic, and non-ionic substances without any appreciable loss of radioactivity. (Analytical grade anion exchange resin, '*AG* 1–*X* 10' (200–400 mesh), chloride form, was obtained from the California Corporation for Biochemical Research, Los Angeles 63. This resin was prepared by sizing and purifying the standard 'Dowex' resin of the same designation by BIO-RAD Laboratories.)

'Dowex 50' (H^+), before use, was treated in bulk with a large quantity of 6 *N* hydrochloric acid and afterwards washed with water until neutral (the *p*H of water). '*AG* 1' (Cl^-) was converted to the formate form in the usual manner by passing *M* sodium formate solution through a burette containing the resin until the effluent was free from chloride ions. The resin was further treated with one resin-bed volume of *N* formic acid and finally washed with water until neutral.

The procedures for the preparation of 'Dowex 50' and '*AG* 1' resin columns and for fractionation are as follows: approximately 10 ml. of the ion-exchanger was poured as a slurry on to a pellet of glass wool in a glass tube, 13 cm. × 1 cm. (intern. diam.), to give a column height of 6·5 cm. After the extract (less chloroform-soluble substances), prepared as described elsewhere[1], was slowly loaded on and allowed to run into the first resin column ('Dowex 50') it was flushed with 15 ml. of water. The rate of flow was maintained at approximately 0·5 ml. per min. Care must be exercised to avoid disturbing the resin surface. The effluent was evaporated *in vacuo* and made up to a known volume. It was then quantitatively transferred to a second resin column ('*AG* 1') following the removal of 10-μl. aliquots for radioactivity assay. The column was flushed with 15 ml. water. This effluent contained non-ionic substances, mainly sugars. The basic substances, essentially amino-acids, on the 'Dowex 50' column were eluted with 25 ml. of *N* hydrochloric acid and followed by 15 ml. of 6 *N* hydrochloric acid and then by 15 ml. of water. Similarly, the acidic substances, mainly non-volatile organic acids, on the '*AG* 1' column were eluted in the same manner but with formic acid. Each fraction was evaporated *in*

Table 1. Fractionation of Alcoholic Extract of Wheat Leaf from Carbon-14 Compound Feeding Experiments and the Recovery of Radioactivity from Ion-exchanger Columns, 'Dowex 50, *X*-8' (H^+) and '*AG* 1, *X*-10' (Formate)

Compound fed	Alcoholic extract (less chloroform-soluble fraction)	Radioactivity (c.p.m.) Fraction						
		Basic	Effluent from 'Dowex 50'	Recovery from 'Dowex-50' (per cent)	Acidic	Non-ionic	Recovery from '*AG* 1' (per cent)	Total recovery (per cent)
Glycine-2-^{14}C	94,700	39,280	52,880	97·5	13,420	34,140	90·0	91·7
,,	94,700	40,290	47,210	92·5	11,910	34,620	98·8	91·7
,,	99,500	37,230	50,260	88·0	6,910	42,830	98·8	87·5
,,	108,700	51,820	59,800	100·0	12,330	44,930	96·0	100·0
,,	176,400	103,030	—	—	19,380	42,240	—	93·7
,,	173,300	116,000	46,160	93·7	16,650	29,160	99·0	94·2
Alanine-2-^{14}C	172,900	144,800	28,000	100·0	17,000	7,500	87·5	97·8
Acetate-1-^{14}C	318,200	78,900	203,920	89·0	176,540	16,430	95·0	85·8

vacuo to dryness. Evaporation was repeated with the addition of water each time until no traces of hydrochloric acid or formic acid remained. The fractions were then made up to a known volume and 10-μl. aliquots were taken for the radioactivity assay. The determination of radioactivity was made with a thin-mica end-window Geiger–Müller counter connected to a Philips scaler, model *PW* 4035. The sum of radioactivity found in each fraction as compared to the initial radioactivity in the alcoholic extract (less chloroform-soluble substances) serves as a means of evaluating the recovery of labelled compounds from each of the ion-exchanger resins. Table 1 shows the recovery of radioactivity from columns of 'Dowex 50' and '*AG* 1'. It is evident from these results that both resins gave reasonably good recovery. Furthermore, little or no ninhydrin-positive substances could be detected in the acidic fraction on paper chromatograms. The method described, therefore, can be satisfactorily used in tracer feeding experiments with plants.

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[1] Wang, D., *Canad. J. Bot.* (in the press).

Gibberellic Acid and Osmotic Pressure

VARIOUS visual responses of the plant to gibberellic acid give reason for thinking that the osmotic pressure of the cell sap is influenced also, for as well the stimulation of cell elongation, paler leaf colour and reduction in slowing-up of growth due to planting out[2] may indicate an improved water-uptake and thus a decrease of the osmotic pressure[1]. In connexion with this an investigation has been conducted with tomatoes, variety 'Moneymaker'.

In an unheated greenhouse, 24 tomato plants were planted on June 12, 1958. Once a week half the plants were sprayed with a solution of 25 p.p.m. 'Gibrel' (the potassium salt of gibberellic acid as produced by Merck and Co., Rahway, New Jersey). The first spraying was carried out on June 17 and the last on August 19. The sprayed and unsprayed plants were planted in a kind of cross-over design.

On six consecutive samplings at weekly intervals material was taken from the plants for measurements of osmotic pressure. The first sampling date was July 4 and the last August 8. Each time two sprayed and two unsprayed plants were taken. From each plant two samples were obtained: one sample consisted of the plant top, 10 cm. in length, and the other one of ten mid-leaf leaflets from the upper ten mature leaves. The samples were kept in a refrigerator at −35° C. in sealed jars for a fortnight and then the material was expressed at a pressure of 175 atm. The osmotic pressure of the press sap was determined cryoscopically with the aid of a thermistor[3]. After each sampling, one sprayed and one unsprayed plant were cut off near the soil surface and weighed. With the rest of the plants a lateral shoot was considered as the new stem. Results of the yield of these plants were recorded until October 1.

The colour of the leaves of unsprayed plants was normal and that of the sprayed ones light green having a grey hue. At the time of sampling the sprayed plants were about 25 per cent longer than the unsprayed ones. This difference was extremely significant ($P = 0{\cdot}001$). However, there was not a significant difference between the plant weights. The first–fourth cluster of the sprayed plants bloomed respectively 0, 2, 2 and 4 days earlier than those of the unsprayed ones. The difference was very significant ($P = 0{\cdot}01$) for the fourth cluster. There was no significant difference between the numbers of flowers and between the numbers of fruit. As a result of a larger mean weight of fruit the total weight of fruit of the unsprayed plants, however, was significantly higher ($P = 0{\cdot}05$) than that of the sprayed ones. The weight of fruit per plant was 2·5 and 1·8 kgm. respectively. For the sprayed and unsprayed plants the average osmotic pressure of the plant tops was 7·9 and 7·4 atm. respectively, and the osmotic pressure of the leaflets 9·3 and 8·8 atm. respectively. Both differences were significant.

So, in the circumstances of this experiment, the application of gibberellic acid did not result in a lower but in a higher osmotic pressure. Evidently the higher osmotic pressure of the leaflets and tops of the sprayed plants is connected with the lower yield, for a lower yield may imply a lesser transport of assimilatory products from the leaves.

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[1] Kramer, P. J., "Plant and Soil Water Relationships" (McGraw-Hill, New York, 1949).
[2] Richards, L. A., and Campbell, R. B., *Soil Sci.*, **65**, 429 (1948).
[3] Zeevaart, J. A. D., *Meded. Dir. Tuinb.*, **21**, 589 (1958).

BIOLOGY

Normal Implantation in Ovariectomized Guinea Pigs

THE presence of the corpus luteum or its hormone is generally regarded as necessary during ovo-implantation in view of the experimental evidence for the effects of ovariectomy on the rabbit, rat, mouse, cat and opossum[1–3]. In 1917, however, Loeb and Hesselberg[4], in a paper on the mammary gland, reported briefly that in four guinea pigs ovariectomy at 3½–6½ days post coitum did not prevent the implantation which normally occurs at 6½–7½ days post coitum. When these animals were killed at 18 days post coitum pregnancy was terminating in three and in one horn of the fourth. Loeb and Hesselberg[4] also stated that implantation occurred in half the guinea pigs if the corpora lutea were cauterized at 3–4 days post coitum, but this work was complicated by the occurrence of fresh ovulations. These experiments do not appear to have been repeated by later writers such as Herrick[5], Nelson[6] and Artunkal and Colonge[7], who examined the effects of ovariectomy on the pregnant guinea pig 14–50 days after insemination and who found that ovariectomy 14–30 days post coitum terminated pregnancy.

In preliminary work on the survival and response to hormones of the unimplanted guinea pig blastocyst, well-developed living fœtuses were found in

guinea pigs ovariectomized on days 3 and 5 post coitum and killed on day 24 and day 28 respectively. For the last 11 days only, these had received 10 mgm. of progesterone daily with the addition of 2 μgm. œstrone on the fourth and fifth days of injection, but the size of the fœtuses indicated that implantation must have occurred without obvious delay at about the normal time. Accordingly, 12 guinea pigs were ovariectomized on days 3, 4, or 5 post coitum and were killed at 10–13 days post coitum, having received no exogenous hormone. Eight of these showed normal implantation swellings, but regression had begun in one. Nine similarly treated animals killed 14–18 days post coitum all had implantation sites, most of which were regressing.

Further experiments showed that if similar ovariectomized mated guinea pigs were given 10 mgm. progesterone daily from day 11 post coitum, normal embryos were found at day 21 in 6 out of 10, indicating that implantation and development could proceed satisfactorily in the absence of ovaries and exogenous hormones up to at least day 11 post coitum. Three of the four other animals showed regressing implantation sites.

The only other species in which the blastocysts are reported to implant after ovariectomy is the armadillo[8], where there is a long delay period in the uterus. Loeb[9] suggested that in the guinea pig sufficient corpus luteum secretion has acted on the uterus by 75 hr. post coitum for a limited development of decidual tissue. In the present work implantations have been found in 35 out of 42 three-, four- and five-day ovariectomized animals, and it would seem either that the amounts of ovarian hormones required to sensitize the uterus are exceptionally low in the guinea pig, or that other supplies are available, perhaps from the adrenals.

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[1] Courrier, R., "Endocrinologie de la gestation" (Masson et Cie., Paris, 1945).
[2] Boyd, J. D., and Hamilton, W. J., Marshall's "Physiology of Reproduction", **2**, Chap. 14, 62 (1952).
[3] Mayer, G., *C.R. Assoc. Anat., Paris*, **40**, 28 (1953).
[4] Loeb, L., and Hesselberg, C., *J. Exp. Med.*, **25**, 304 (1917).
[5] Herrick, E. H., *Anat. Rec.*, **39**, 193 (1928).
[6] Nelson, W. O., *Endocrinol.*, **18**, 33 (1934).
[7] Artunkal, T., and Colonge, R. A., *C.R. Soc. Biol., Paris*, **143**, 1500 (1949).
[8] Buchanan, G. D., Enders, A. C., and Talmage, R. V., *J. Endocrinol.*, **14**, 121 (1956).
[9] Loeb, L., *Amer. J. Anat.*, **32**, 305 (1923).

Response of Larvæ of *Calyptraea chinensis* (L) to Light

The veliger stage of the prosobranch *Calyptraea chinensis* is passed in capsules fixed to the substratum and guarded under the fore part of the shell of the parent. The young hatch as crawling post-veliger larvæ. During studies on the reproduction of this animal at Plymouth, embryos of all stages were examined at night in sea water in a glass dish lit only by a hooded microscope lamp. Freed veliger larvæ of stages 5 and *5a* (unpublished work) were seen to mass in the illuminated part of the dish facing the light. The early and middle larvæ swam well; many of the late veliger larvæ alternately swam and crawled. The later veliger larvæ, which had been ready to hatch, crawled only. When the position of the light was changed, the veligers swam or crawled towards the light. Other newly liberated marine larvæ show a similar response. Larvæ of *Lepidochitona cinereus* aggregate in regions of low light intensity[1] and larvæ of *Spirorbis borealis* are photopositive for up to 2 hr.[2]. The nauplii of barnacles are generally photopositive[2] and the cyprids also[3]. The response to incident light by the early and middle veliger larvæ of *Calyptraea* is interesting as the larvæ are never free-swimming and are enclosed under the shell where there is little light.

Hatched larvæ kept in glass finger bowls under circulating sea water were active, crawling over the bottom and lower sides. When the animals were about 1 mm. shell diameter (about 9 days after hatching), a glass slide was placed in a bowl, resting about 2 cm. from the bottom. Although a few animals remained on the bottom of the bowl, most congregated on the under-side of the slide, especially where it touched the sides of the bowl. None was seen on the top of the slide. When an empty *Paphia* shell was placed in a bowl containing newly hatched animals and others 1 week old, most were found next morning on the under-side of the shell, where they stayed. In the River Yealm near Plymouth, small *Calyptraea* of less than 2-mm. shell diameter were found on the under-side of empty *Paphia* shells. Adults, however, were found mostly on the upper surfaces of stones, shells and glass, to which they are confined by their need for a smooth surface.

It appears from these observations that although the retained veliger larva would be positively phototropic if free, the hatched animal crawls until it finds the under-side of a surface whether dark or illuminated. Adults are found on any smooth surface.

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[1] Matthews, G., *Brit. J. Anim. Behav.*, **1**, 79 (1953).
[2] Knight-Jones, E. W., *J. Mar. Biol. Assoc. U.K.*, **30**, 201 (1951).
[3] Barnes, H., Crisp, D. J., and Powell, H. T., *J. Anim. Ecol.*, **20**, 227 (1951).

A Red Water Bloom of Rotifers in Cook Strait, New Zealand

On September 11, 1959, during an oceanographic cruise by M.V. *Viti* in Cook Strait, the sea surface was observed to display a blood-red discoloration in large, streaky patches over a stretch of 2–3 miles. Two samples were collected by Dr. D. E. Hurley from the surface in a phytoplankton tow net made of No. 25 bolting nylon: one 10 miles south-west of Kapiti Island (1603–1610 hr., 41° 01′ S., 174° 28′ E.—N.Z. Oceanographic Institute Station *C* 201), the second about 2 miles farther south (1708–1735 hr., 41° 04′ S., 174° 31′ E.—N.Z. Oceanographic Institute Station *C* 203). The latter was deep-frozen, the former preserved in 5 per cent neutral formalin.

Six days later, microscopic examination in the laboratory showed the dominant organisms to be rotifers. In the formalin-preserved sample, ctenophores (identified by Miss P. M. Ralph, Victoria University of Wellington, as *Pleurobrachia pileus* Vanhöffen) occurred in large numbers; but in the deep-frozen sample rotifers were the sole dominants. Both samples were collected from areas where the red colour of the water was pronounced. Mr. C. R. Russell (Canterbury Museum, Christchurch) has con-

firmed that the rotifers belong to the genus *Synchaeta*. Of the three species present, *S. baltica* Ehrenberg appeared to be most common, with ?*S. stylata* Wierzejski in moderate numbers and *S. curvata* Lie-Pettersen of rare occurrence. *S. baltica* is known to be abundant at times in the open sea (Rousselet[1]). A red colour, found in the ganglion and eyespot, is typical of the genus *Synchaeta*. Other species in the samples were :

C 201 (5 per cent neutral formalin)

Pleurobrachia pileus Vanhöffen	very abundant
Polychæte larvæ	occasional
Rhizosolenia styliformis Brightwell	occasional
Oscillatoria thiebautii (Gom.) Geitl.	rare
Spore of *Goniaulax digitale* ? (Pouch.) Kofoid (ident. E. J. F. Wood)	rare
A tintinnid : *Epiplocylis acuminata* Jorg. (ident. E. de Sousa e Silva)	rare
C 203 (deep-frozen)	
Rhizosolenia styliformis Brightwell	scarce
Ceratium furca (Ehr.) Clap. and Lach.	scarce
Cerataulina pelagica (Cleve) Hendey	rare
Spore of *Goniaulax digitale* ? (Pouch.) Kofoid	rare
Epiplocylis acuminata Jorg.	rare

Immediately after the fresh sample was placed in a 1-lb. jar, a mass of red-coloured organisms, each about the size of a pin-head, was seen by Dr. Hurley to congregate about the surface of the sea water. While there is a possibility that the blood-red discoloration might have been caused by one of the other plankton organisms in the samples, the evidence in favour of *Synchaeta* seems more conclusive. It could be argued that a smaller dinoflagellate like a *Goniaulax* spore was initially present in large quantities, but lost in the preservative. Against this, the spore was very scarce in the deep-frozen sample. Also, Braarud[2] found a *Gymnodinium* (*G. galatheanum*) dominant in a formalin-preserved sample collected by the *Galathea* in Walvis Bay, South-west Africa, on December 24, 1950. The fact that the dinoflagellate spore was rare in the Cook Strait material would seem to indicate that it did not contribute significantly to the strong colour. Bary[3] has recorded discoloured water from this area, but in no case were rotifers cited as forming a visible bloom. Further, no mass mortality of fish or other organisms has been reported as a result of this bloom.

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[1] Rousselet, C. F., *J. Roy. Micro. Soc.*, **3**, 269; **4**, 393 (1902).
[2] Braarud, T., Galathea Report, **1**, 137 (1957).
[3] Bary, B. McK., *N.Z. J. Sci. and Tech.*, B, **34**, 393 (1953).

Recovery of Adult *Amoeba lescherae* from Dried Cultures

IN 1950, Galbraith and Taylor[1] obtained adult *Amoeba proteus* from the brown powder formed when a culture of that species was allowed to dry. Galbraith has repeated the experiment; but hitherto, attempts to recover adults of *A. lescherae* from dried material have failed. A detailed description of these experiments has already been given[2,3] and the behaviour of developing *Amoebae* of the *proteus*-type[4] fully portrayed. This communication announces the successful recovery of adult *A. lescherae* by the following method.

Large numbers of young adults were introduced into 'Petri' dishes, 4 in. diameter, where they were allowed to increase by fission-divisions, and so, gradually, to age. The increasing size of the nutritive spheres (= excretion spheres = refractive bodies) is indicative of the approach of the sporulation cycle; when they measure not less than 3μ in diameter the *Amoebae* are old enough to produce spores. It must be emphasized that only spores and encysted stages of development (see below) can withstand the vicissitudes of drying. An adult *Amoeba* (that is, of the *proteus* type[4]) does not form any protective covering for itself; it disintegrates as the culture dries up.

These cultures (well stocked with food organisms) consisting of mature *Amoebae* ready to sporulate are exposed to the air until completely dry. They are then covered with a bacteria-rich fluid. After a few days an examination of the 'Petri' dishes with a 4·4-mm. water immersion lens reveals the presence of amoebulae 5–6μ long hatching out of the spores. These feed on bacteria and grow. After a time, however, they encyst, remain quiescent and eventually excyst. Feeding follows, with a consequent increase in size, and again the creatures encyst. This phenomenon proceeds, with cysts measuring respectively 10–15–20μ in diameter. From cysts 25μ in diameter young adults emerge very transparent because of the few cytoplasmic contents. They feed voraciously, undergo fission and grow. The time required is 6 months.

Here it must be explained that, except under the most rigid control, no culture of *Amoebae* is exactly homogeneous as regards age. Especially is this true of cultures produced by method I[3] or cultures obtained in Nature. Among the progeny of a single *Amoeba* some individuals race on and attain maturity before the rest. These sporulate, thus producing young encysting stages of development (see above) and these lie in the culture along with the rest of the inhabitants. It is therefore probable that in the experiments described above, because of the presence of encysted young, the first examination of the dried-up and wetted culture may reveal larger amoebulae than those hatching from the spores.

During the routine examination of the cultures which were recovering from the effects of desiccation I found 'activity' after some hours of daylight. In the early morning, cysts were more common than active *Amoebae*.

I have not succeeded in stemming the onset of encystment nor of shortening its duration. Galbraith (private communication) kept cysts of about 10μ diameter under observation for two years, after which they excysted and fed.

Under natural conditions this power to encyst possessed by the large free-living *Amoebae* of the *proteus*-group is clearly an adaptive character.

Spores are produced in late autumn or early winter —the onset of a favourable environment may cause the spores to hatch prematurely; a sudden return to winter conditions, lack of food, low temperature, desiccation would kill off the delicate amoebulae. Hence the necessity of this power to encyst at all levels of development. But it is difficult to understand why this characteristic cannot be broken down under laboratory conditions which can be adjusted to the needs of the *Amoebae*.

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[1] Galbraith, M., and Taylor, M., *Nature*, **165**, 938 (1950).
[2] Taylor, M., Hayes, C., and Galbraith, M., *Proc. Roy. Soc. Edin.*, **66**, Part 3, No. 15 (1957).
[3] Taylor, M., *School Science Review*, No. 136 (June 1957).
[4] Jepps, M. W., "The Protozoa, Sarcondina" (Oliver and Boyd, Edinburgh, 1956).

MICROBIOLOGY

Plaque-forming Ability of Urea- or Phenol-treated T_2 Coli-Phage Preparations

The treatment of T_2 phage with urea was used by Cohen[1] to prepare deoxyribonucleic acid of T_2 phage. Mahler and Fraser[2] also liberated T_2 deoxyribonucleic acid by urea treatment and described the infection of *E. coli* lysozyme-protoplasts by it.

I investigated the survival of urea- or phenol-treated T_2 (or T_2rh) phages by their ability to form plaques when they are mixed with cells of *E. coli*.

In general, the standard bacteriophage techniques as described by Adams[3] were used. Special methods were used as follows. *Urea treatment:* purified T_2 bacteriophage at 10^{11}–10^{12} particles/ml. were diluted 1 : 10 in a solution of 8 *M* urea and 0·1 *M* sodium chloride, adjusted to *p*H 8. After incubation at 37° C. for 30–90 min., the preparation was dialysed in the cold for 16–24 hr. against water, phosphate-buffer (0·1 *M*, *p*H 7) or sodium chloride (0·1 *M*). The dialysis fluid was changed after 12 hr. At the end of this time, the urea concentration was lower than 0·001 *M*. *Phenol treatment:* 5×10^{12}–10^{13} phage/ml. were diluted 1 : 10 in a solution of 3 per cent phenol and phosphate-buffer (0·1 *M*, *p*H 6·5) at 4° C. After 30 min. the mixture was dialysed in the same manner as the urea preparation.

Preparation of bacteria: The cells were grown up to 1–5 × 10^9 cells/ml. in a medium containing 0·5 per cent sodium chloride and 1 per cent peptone or nutrient broth (Difco). The cells were used in this form as indicator-cells or washed twice with the media described below and brought up to 5 × 10^9 cells/ml. Usually, 0·1 ml. of this cell suspension was incubated with 0·5 ml. of the urea (phenol) preparation or with a dilution thereof. After incubation at 20° C. for 10 min. the mixture was plated by pouring it with 3 ml. soft agar on solid agar plates. The rate of survival of the urea-treated phage changed under the experimental conditions very markedly according to the suspension-medium of the cells. A urea preparation of the initial titre of 10^{12} phages/ml. gives with indicator cells 0–10^3 plaques/ml. Starting with a urea preparation giving 200 plaques/ml., we find a notable increase of the titre to more than 10^4, incubating with bacteria, washed and suspended in: (*a*) broth, 1 per cent peptone, 0·5 per cent sodium chloride (titre ~ 200); (*b*) water (titre ~ 6 × 10^3); (*c*) water–sucrose 13 per cent pl 60 (titre ~ 10^4); (*d*) 0·1 *M tris* buffer *p*H 8 (titre ~ 2 × 10^4); (*e*) 0·1 *M tris* buffer *p*H 8 enriched with sucrose 13 per cent (titre ~ 8 × 10^4).

A supplementary 5–10 fold increase was obtained with bovine serum albumin, added to the suspension media (*a*–*e*). The plating efficiency increases 3–10 fold, when using sucrose (13 per cent) – soft agar instead of the usual soft agar for pouring the incubation mixture on the agar plates. Altogether, we find a titre increase of more than 10^5. It means, in general, a difference of 10^4 between the highest and lowest titres obtained.

The increase of the rate of survival is not observed when an incubation mixture as described under *a* is supplemented with a fifth volume of water–sucrose, *tris*, or *tris*–sucrose solution as described under *b*–*e*.

The plaque-forming particles are adsorbed at *E. coli B* cells, as shown by the following experiment. When, at the end of the incubation time, the cells are removed from the incubation mixture by low-speed centrifugation ~ 90 per cent of the infective centres are found in the pellet. In the supernatant, which is incubated a second time with *E. coli B* cells, only ~ 10 per cent of the plaque-forming particles are re-detected.

It is of special interest that *E. coli B*/2 instead of *E. coli B* cells were used with similar results. In a larger series of experiments, *B*/2 cells, suspended in *tris*–sucrose, were incubated with urea-treated phages and plated together with indicator cells of *E. coli B*. The increase in rate of survival was between 10 and 200 per cent, as compared to the results obtained in the experiments described above.

The rate of survival obtained with bacteria suspended in *tris*–sucrose falls to 1/10–1/100 of its original value after preincubation with trypsin, chymotrypsin, subtilisin or papain (50 γ/ml.) for 20 min. at 37° C. and a *p*H of 7·5, whereas the titre of a suspension of 10^5 phages/ml. is not notably changed under the same conditions. The survival-rate is also reduced by T_2 antiserum. However, the rate of inactivation is probably less than that of intact phages.

In the analytical centrifuge deoxyribonucleic acid liberated by the urea treatment is represented by a high peak. Despite the disappearance of this peak after incubation with deoxyribonuclease (50 γ/ml., *p*H 7, 20 min., 37° C.), the rate of survival is changed in varying degrees. There can be a small increase as well as a decrease to a fifth of the value.

With respect to these properties of the urea-treated phage preparation, it is difficult to say what kind of infective agent is involved. It may be due to phage heads with partly or wholly removed tails.

Similar results, but with a lower increase of the titre, have been obtained with phenol preparations.

The details of this and further investigations of this problem will be published elsewhere.

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[1] Cohen, S. S., *Cold Spring Harbor Symp. Quant. Biol.*, **12**, 35 (1947).
[2] Fraser, D., Mahler, H. R., Shug, A. L., and Thomas, jun., Ch. A., *Proc. U.S. Nat. Acad. Sci.*, **43**, 939 (1957).
[3] Adams, M. H., "Bacteriophages" (New York, 1959).

Induction and Reversion of *c*-Mutations by Irradiation of the Extracellular ϰ-Phage of *Serratia*

It was reported earlier[1] that mutations to clear-plaque type are induced by ultra-violet irradiation of the ϰ-phage in the extra-cellular state (without irradiation of the host bacteria) up to about 2 per cent as a two-hit process. Such *c*-mutations appear also when the same phage is irradiated as prophage of the lysogenized *Serratia* strain HY_2(ϰ) (ref. 2). Since the rate of induced mutation in this intracellular prophage is about the same as in the free phage, 'indirect' effects of ultra-violet radiation via the intracellular environment do not seem to play an essential part in this mutation-induction process. Postcultures of single fresh mutant plaques show pure mutant type in most cases; therefore, both chains of the deoxyribonucleic acid seem frequently to be changed by the

mutation induced by ultra-violet irradiation. A few postcultures gave a mixture of *c* and normal plaques, and the second postcultures of these *c* plaques were pure *c*. Thus, a kind of 'heterozygous' segregating state, probably due to change of only one base partner in the deoxyribonucleic acid double chain, seems to be caused by some of the mutating events.

c-Mutations are also produced in the free phage by X-rays (50 kV., no filter, 65,000 r./min.). The dose curve for killing is (as with ultra-violet radiation) a one-hit function; the *c*-mutations are induced by one hit, too. Selection of spontaneous *c* mutants by irradiation was excluded for X-rays as well as ultra-violet radiation. The formal target size for killing is 4,000 mμ[2] (the 37 per cent dose is 145,000 r.); the target for *c*-mutations appears about 5,900 times smaller. When several stable *c* mutant strains induced by X-rays were X-irradiated, 'back'-mutations to the (turbid, lysogenizing) wild type appeared in some of them at a rate of a few per thousand. Some of the ultra-violet-induced *c* strains produce a few back mutations spontaneously. This may indicate that many of the *c*-mutations are not deficiencies.

In order to examine whether the mutational 'lesions' produced by ultra-violet radiation in the phage are reversible 'pre-mutations' (as in irradiated cells), post-treatments of the free phage after ultra-violet irradiation by visible light or heat were done. When the irradiated phage was illuminated up to 1 hr. in the free state (before infection) at 30° C. by white daylight fluorescent tubes, the frequency of *c*-mutation decreased strongly and about proportionally to the light dose from 13·9 to 4·9 per thousand. When Osram photocopy lamps (giving mainly blue to long-wave ultra-violet light) were used, the maximum photo-reversion of about half the mutations was already obtained after 8 min. Higher light doses caused a further increase though this light alone was not mutagenic. Survival was only slightly affected, for example, from $6{\cdot}3 \times 10^{-3}$ to $7{\cdot}9 \times 10^{-3}$ at 8 min. *PC*-light. In an experiment where the ultra-violet-irradiated phage was mixed with host bacteria for ½ hr. and then illuminated after infection, the photoreversion of the *c*-mutations was much smaller (from 13·9 to 10·7 per thousand after 60 min. of white light), but the photoreactivation of killing was much higher (from a survival of $2{\cdot}0 \times 10^{-3}$ to $1{\cdot}9 \times 10^{-2}$) than in the extracellular phage. From these findings it can be concluded that photoreversion of mutation induced by ultra-violet light is possible, and even better, in extracellular phage without participation of metabolic processes of the cell, but that for photoreactivation of killing the condition of the cell interior, perhaps its metabolism, is an important factor. Obviously, the lesions induced by ultra-violet radiation in the free phage leading to *c*-mutations are not yet perfect mutations, but photoreversible 'pre-mutations' residing most probably in the phage deoxyribonucleic acid. This strongly supports the view[3] that in cells, for example bacteria, the photoreversible step of the mutation process is a state within the deoxyribonucleic acid and not a step in a reaction chain outside the genom as assumed by the hypothesis of 'indirect' mutagenic action of ultra-violet light (via a changed cell metabolism or production of organic peroxides, etc.).

Reversible chemical changes induced by ultra-violet light in deoxyribonucleic acid had been indicated by Sinsheimer's findings[4] that the absorption spectrum of cytosine (not of thymine or purines) changes after irradiation with ultra-violet light and that this change can be reversed by heat. In order to examine whether the pre-mutational ultra-violet lesions in the phage could be a change of this kind in the deoxyribonucleic acid, post-treatments with high temperature up to 1 hr. in the dark after ultra-violet irradiation of the free phage were carried out. The experiments at 30° C. showed only an insignificant decrease in production of *c*-mutations; but at 40° C. a big decrease of the frequency of mutation from 8·0 to 4·5 per thousand after 30 min. was observed; survival increased but not significantly (from $6{\cdot}2 \times 10^{-3}$ to $7{\cdot}5 \times 10^{-3}$). This heat reversion of pre-mutations due to ultra-violet irradiation of the free phage suggests that the ultra-violet lesions in the phage are of a similar nature to the changes causing the decrease in the absorption spectrum of nucleotides, which are probably due to addition of water at a double bond within the cytosine ring[4]. Since the high mutability induced by irradiation of the free κ phage is exceptional compared with other phages (*T*-series or λ of *E. coli*), it is probable that the deoxyribonucleic acid of this phage is in a special state which permits the induction of pre-mutational lesions or their 'survival' until reproduction takes place.

Because only clear plaque mutations are induced at a high rate and other types of mutation, for example, with changed colour halo of the plaque, are very rare, only the *c* region of the genom seems to be in the special mutable state. This is supported by the observation that most of the *c*-mutations occur in the same cistron, because no phenogenetic co-operation for lysogenization between different *c* mutants was found using Kaiser's method[5] in cross-tests among 7 X-rays- and 5 ultra-violet-induced *c* mutants in all combinations.

The results will be published in detail elsewhere. The work was supported by the Deutsche Forschungsgemeinschaft. We thank Mrs. H. Stoye and Miss G. Dittrich for technical assistance.

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[1] Ellmauer, H., and Kaplan, R. W., *Naturwiss.*, **46**, 150 (1959).
[2] Ellmauer, H., Diss. Univ. Frankfurt am Main (1959).
[3] Kaplan, R. W., *Exp. Cell. Res.*, **11**, 378 (1956). Kaplan, R. W., and Gunkel, W., *Arch. Mikrobiol.* (in the press). Goes, E., and Kaplan, R. W., *Naturwiss.*, **46**, 335 (1959).
[4] Sinsheimer, R. L., *Rad. Res.*, **6**, 121 (1957); **1**, 505 (1954).
[5] Kaiser, A., *Virology*, **3**, 42 (1957).

Pigment-producing Strains of *Acetobacter aceti*

Asai and Shoda[1] have recently proposed a new classification of the acetic acid bacteria, basing this on a study of 26 strains. They have substantially supported Leifson's findings[2] (confirmed by Shimwell[3]) that these bacteria are not a homogeneous group, but consist of two distinct genera, the first (*Acetobacter* proper) oxidizing ethanol to acetic acid and thence to carbon dioxide and water and possessing, if motile, peritrichous flagella; the second (which Leifson[2] called *Acetomonas*) oxidizing ethanol only as far as acetic acid and having, if motile, polar flagella.

However, Asai and Shoda[1] have rejected Leifson's name *Acetomonas* on the grounds that their strains

produced little or no acetic acid from ethanol, and have proposed their own name '*Gluconobacter*' because such strains produce relatively large quantities of gluconic acid from glucose.

Two of their *Gluconobacter* strains, however (*G. melanogenus AC–8* and *U–4*), did not conform with their own diagnosis of *Gluconobacter*, as they oxidized acetate to carbonate. These strains also produced a dark brown water-soluble pigment on glucose media, thus resembling *Acetomonas melanogena* (*Acetobacter melanogenum* Beijerinck).

By the courtesy of Dr. T. Asai we have examined these apparently anomalous *Gluconobacter* strains and we have found them to have, not polar, but peritrichous flagella[4], Liefson's correlation between polar flagellation and failure to oxidize acetate still holding good. Our finding has been confirmed by Dr. E. Leifson and Dr. A. H. Stouthamer, working independently (private communications).

Furthermore, we have now typed these strains in accordance with Frateur's[5] criteria and have found them to be not only strains of *Acetobacter* but also of the type species of that genus—*A. aceti*.

Their production of dark brown water-soluble pigment is, however, indeed novel and anomalous; we know of no other strains of *Acetobacter* possessing this property which, among the acetic acid bacteria, has previously been the monopoly of *Acetomonas melanogena*.

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[1] Asai, T., and Shoda, K. J., *J. Gen. App. Microbiol.*, **4**, 289 (1958).
[2] Leifson, E., *Antonie van Leeuwenhoek*, **20**, 102 (1954).
[3] Shimwell, J. L., *Antonie van Leeuwenhoek*, **24**, 187 (1958).
[4] Shimwell, J. L., and Carr, J. G., *Antonie van Leeuwenhoek*, **25**, 353 (1959).
[5] Frateur, J., *La Cellule*, **53**, 287 (1950).

A Micromethod for testing for Niacin

The direct chemical method described by Konno[1,2] for routine differentiation of human tubercle bacilli from other *Mycobacteria*, based on the fact that human strains produce larger amounts of nicotinic acid, is difficult because it demands a relatively large amount (at least 20 mgm.) of bacteria[3–5]. I have worked out a method which needs only a small quantity of bacteria (about 1 mgm.). I found benzidine more suitable than aniline, as used by Konno, for testing for nicotinic acid qualitatively. This reagent gives a violet pink compound with cyanogen bromide and nicotinic acid, which stains the precipitating benzidine crystals. Its sensitivity is four times that of the aniline reagent.

For the micromethod the following simple equipment is used: thin conical tubes (55–65°) of 16 mm. diameter as used for centrifuging of sediment from urine; Pasteur pipettes giving 9–12 mgm. drops of reagents; rather thick platinum wire in a loop-holder; the end of the wire is flattened to make a microspatula for handling the bacteria; thin glass rods (rounded ends).

As reagent I used a 3 per cent alcoholic solution of benzidine (freshly crystallized reagent grade) in a brown glass bottle at 0–4°, which will keep for weeks, and a concentrated aqueous solution of cyanogen bromide (about 10 per cent) which can be used for several months if kept in a refrigerator.

For the examination of about 1 mgm. bacteria, drop 9–12 mgm. of benzidine solution with a Pasteur pipette to the bottom of a conical tube and add the bacteria (Löwenstein-Jensen solid medium) with the microspatula to the fluid and homogenize it with a glass rod. About the same weight of cyanogen bromide is then added. The bacteria are stained a faint pinkish colour and the benzidine crystals a violet-pinkish colour either at once or within 10 min. The effects are examined with a magnifying glass in reflected daylight.

As control for the reagents, one drop of benzidine solution and one drop of cyanogen bromide solution are mixed and should remain colourless for at least 10 min. As bacterial controls, add one drop of both reagents to about 1 mgm. of bacteria from a bovine strain (aged 35–50 days) recently cultivated on Löwenstein-Jensen medium. For checking the activity and colouring intensity of the reagents, it is advisable to use a similar control with a human strain, cultivated recently on Löwenstein-Jensen medium.

I have checked the procedure with the saprophytic *Mycobacteria* strains (*M. butyricum*, *M. pellegrini*, *M. frieburg*, *M. phlei*, *M. rabinowitsch*), on four laboratory bovine strains, on two *BCG* strains, on two avian strains, all giving negative reactions (or sometimes a weak one) and on eight laboratory human strains (very strong reactions), and also with three strains resistant to niacin and two resistant to streptomycin, which gave strong reactions (Löwenstein-Jensen cultures). Besides these, I tested 247 routine Löwenstein-Jensen cultures of which five gave negative reactions; and a further 94 routine Sula cultures of which two gave negative reactions. (All tubes were placed in a boiling water-bath for 15 min. before testing.)

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[1] Konno, K., *Science*, **124**, 985 (1956); *Beitr. Klin. Tub.*, **119**, 1 (1958).
[2] Konno, K., Kurzmann, R., Bird, K. T., and Sbarra, A., *Amer. Rev. Tuberc.*, **77**, 669 (1958).
[3] Bönicke, R., Jahresbericht 1956–57 des Tuberkuloseinstituts Borstel.
[4] Marek, A., *Beitr. Klin. Tuberc.*, **117**, 529 (1957).
[5] Gilani, S., and Selkon, J. B., *Tubercle*, **39**, 396 (1958).

CYTOLOGY

Cytoplasmic Inclusions of the Ageing Neurons of some Teleosts

The spinal ganglion cells show distinct ageing phenomena, not only in man and mammals, but also in amphibians[1]. As to the best of our knowledge no data are available concerning the ageing phenomena of the spinal ganglion cells in fishes, I have studied the cytoplasmic inclusions of these neurons in the oviparous cyprinodont *Aphyosemion coeruleum*, the viviparous cyprinodonts *Lebistes reticulatus*, *Xiphophorus helleri* and *Xiphophorus maculatus*, the hemiramphid *Dermogenys pusillus*, the cyprinid *Brachydanio rerio*, the characid *Hyphessobrycon innesi*, the cichlids *Haplochromis multicolor* and *Hemichromis bimaculatus*, the anabantid *Betta splendens* and the erythrinid *Nannostomus beckfordi*, employing various

techniques, namely, 0·5 per cent iron hæmatoxylin after Champy, Helly post-chromed and Regaud post-chromed fixation; 1 per cent solution of toluidine blue in water after Carnoy fixation; 1 per cent aqueous solution of nile blue after Regaud post-chromed fixation; silver nitrate technique of Aoyama; osmium tetroxide techniques of Mann-Kopsch and Baker[2]; Sudan black after the techniques of Thomas[3] and Baker[2]; sudan black after Aoyama fixation without silvering, and silvering followed by post-chroming according to the method of Lacy[4]; sudan IV saturated solution in 70 per cent alcohol after formaldehyde-saline fixation[1] and Helly post-chromed fixation (staining at 37° C. as recommended by Gatenby and co-workers[5]); 0·02 per cent solutions of methylene blue, neutral red and janus green; phase-contrast technique.

In this investigation the spinal ganglia of young, middle-aged and old fishes were studied. The observations made on the fixed material were supported by examination of corresponding living neurons by the phase-contrast technique. This phase-contrast examination also included an account of freshly teased spinal cord neurons examined with positive phase-contrast.

In the neurons of all the specimens, discrete spheroidal bodies, vesicular and homogeneous, duplex in structure, were prominently present. These spheroidal bodies have been homologized with the lipid spheroids of the invertebrate neurons. In the neurons of the adult fish a chromophil product is formed in the chromophobe core of the duplex spheroidal bodies.

The present results on the shape and the structure of the lipid spheroids are an argument for the theory of Golgi structure of Hirsch[6], according to which the homogeneous granules are considered as dynamic bodies, the so-called 'Praesubstanz' granules. Afterwards these granules grow and differentiate into true Golgi systems, each consisting of an inner chromophobe Golgi internum and an outer chromophil Golgi externum. The Golgi systems of Hirsch and the duplex, lipid spheroids of Malhotra[1] in *Rana tigrina* and also in the teleost species examined appear to be strictly comparable to the plaquettes found by Golgi[7] interposed in the meshes of the Golgi apparatus.

In general, the cytoplasmic inclusions of the ageing neurons in the teleost species examined correspond very well with those in *Rana tigrina*[1].

Nissl bodies, varying in shape and size from small granules to big, irregular structures, occurred in the neurons of fixed preparations of old specimens. In life the Nissl substance most probably exists in a dispersed state, for in living neurons no Nissl bodies could be identified by the phase-contrast technique. This result is in conformity with the findings concerning the spinal ganglion cells of the rat[8] and *Rana tigrina*[1].

Mitochondria appeared to be present as filaments and granules, which were fairly regularly dispersed throughout the cytoplasm of the neurons of all the specimens. Some of the filamentous mitochondria appeared to be quite short, rod-like and small.

Even in the neurons of the young fish no canalicular Golgi reticulum of Gatenby and co-workers[5] could be demonstrated. Consequently, the possibility of the spheroidal bodies being considered as having originated from the canalicular Golgi reticulum is ruled out. No aggregation of the granular homogeneous spheroids was found at the axonal pole in the neurons of even the oldest specimens.

Canals of Holmgren, as described in the neurons of toads and *Pheretima*[1], were not present in the neurons. These canals were also absent from *Rana tigrina*[1].

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[1] Malhotra, S. K., *Nature*, **176**, 886 (1955); *Quart. J. Micro. Sci.*, **97**, 177 (1956); **98**, 65 (1957); *Curr. Sci.*, **29**, 157 (1957); *La Cellule*, **58**, 365 (1957); *Res. Bull. Panjab Univ.*, **118**, 123 (1957).

[2] Baker, J. R., *Quart. J. Micro. Sci.*, **85**, 1 (1944); **90**, 293 (1949); *Proc. Linn. Soc. Lond.*, **162**, 67 (1950); *Bull. Micro. App.*, **3**, 1 (1953); *Nature*, **172**, 617 (1953); *J. Roy. Micro. Soc.*, **74**, 217 (1954); *Soc. Exp. Biol.*, **10**, 1 (1957).

[3] Thomas, O. L., *Quart. J. Micro. Sci.*, **88**, 445 (1947); **89**, 333 (1948); *J. Comp. Neurol.*, **95**, 73 (1951).

[4] Lacy, D., *Quart. J. Micro. Sci.*, **95**, 163 (1954).

[5] Gatenby, J. B., *et al.*, *J. Roy. Micro. Soc.*, **73**, 61 (1953); **69**, 185 (1949); **70**, 342 (1950); *La Cellule*, **53**, 13 (1949); **55**, 137 (1953).

[6] Hirsch, G. C., quoted from Nath, V., 31st Indian Science Congress, Delhi (1944).

[7] Golgi, C., *Arch. ital. de biol.*, **30**, 60 (1898), quoted from Baker, *Nature*, **172**, 617 (1953).

[8] Beams, H. W., *Anat. Rec.*, **49**, 309 (1931).

Effect of Centrifugation on the Macronucleus of *Spirostomum* and *Blepharisma*

In a recent communication[1] it was reported that the macronucleus of *Spirostomum* (Ciliophora : Spirotricha) could be mechanically pulled to a great length and was therefore highly extensile. That a nucleus could be manipulated to the extent of being stretched to a length fifteen times the original is interesting enough, but the findings of further experimental treatment of this and another ciliate belonging to the same family, *Blepharisma*, are even more so. The present communication briefly describes them.

The macronucleus in both genera is a large body extending over the greater part of the length of the animal. It is intensely Feulgen positive and in the vegetative condition shows a limiting membrane within which particles of different sizes can be distinguished[2]. The experimental treatment consisted in subjecting a few hundred animals in a centrifuge tube to centrifugation at 1,855*g* for varying periods of time. The material was then fixed in Carnoy's fluid and stained with the Feulgen reagent.

In animals centrifuged for 5–10 min., the macronucleus is precipitated in a clump towards one end of the animal. Over slightly longer periods, the cytoplasm breaks up, liberating the macronucleus which now becomes pulled out into a more or less uniform body in much the same way as occurs when it is stretched mechanically[1]. Centrifugation for 15–20 min. results in further elongation. Close exam-

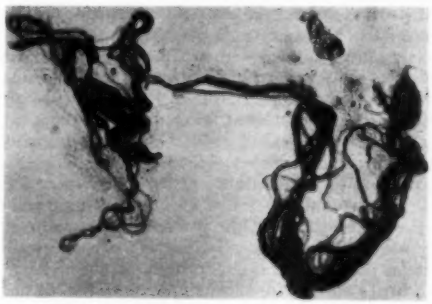

Fig. 1. Macronuclear substance from *Spirostomum* sp. obtained from centrifugation of animals for 45 min. at 1,855*g*. Feulgen. (× 460)

Fig. 2. Macronuclear substance from *Blepharisma undulans*. Centrifugation for 45 min. at 1,855*g*. Feulgen. (× 370)

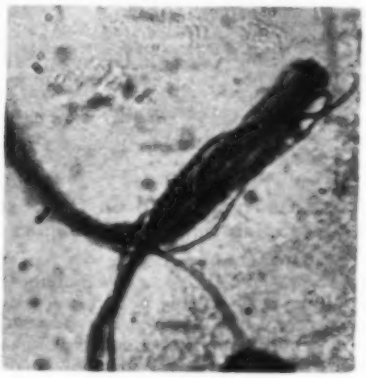

Fig. 3. A portion of the macronuclear material of *B. undulans* under higher magnification showing bundle of fibres. Feulgen. (× 1,840)

ination shows that the macronucleus has lost its original particulate appearance and is now a bundle of parallel fibres. Some of these attain an incredible length as well as tenuity, the latter almost at the limits of resolution of the light microscope. The number of fibres is also very large. Continuous centrifugation for 45–60 min. results in the resolution of the entire macronucleus into a mass of fibres in which no part of the original structure can be made out. The fibres remain Feulgen-positive throughout.

The only possible interpretation of these findings seems to be that the macronucleus of ciliates consists of deoxyribonucleic acid protein essentially fibrous in nature. The particulate and granular appearance of the macronucleus in fixed preparations is evidently an artefact and the granules could very well represent regions where the filaments are tightly coiled and which, under the centrifugal force, become resolved into their component fibrous system. It will be recalled that Ris[3] has recently presented evidence, not only in the mitotic chromosome but also in the salivary gland chromosome, that the 'chromomere' is nothing but the region where the chromosome fibrils (in this case, microfibrils) have been folded over repeatedly to present the appearance of a deeply staining body. It is not unlikely that the granules of the macronucleus are also of the nature of folded fibrils.

This has an important bearing on the general problem of the structure of the ciliate macronucleus and the question whether it contains chromosomes. The macronucleus is, in all ciliates where conjugation occurs, a product of development of the synkaryon, which in its turn is formed from micronuclear derivatives. Increase in size has been associated with loss of mitotic ability and obscuration of chromosomes, and conjectures as to the nature and significance of the differences between the micronucleus and macronucleus have been numerous. Indirect evidence that the macronucleus is either polyploid or compound has been repeatedly presented: observations of threadlike bodies in the macronucleus have been quoted as evidence of chromosomes. The present observations of the occurrence of Feulgen-positive filaments in the macronucleus show conclusively the essentially chromosomal nature of its material.

The precise nature of these filaments is, however, difficult to determine. Their great length would appear to present difficulties in regarding them as individual chromonemata. The possibility that they could represent the greatly extended state of the deoxyribonucleic acid protein fibres[4] would also have to be considered. Reconciliation of the difference between the appearance of the vegetative macronucleus and that presented on centrifugation does not appear possible at the level of the light microscope; perhaps other physical and chemical methods will provide a clue.

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[1] Seshachar, B. R., *Nature*, **182**, 1614 (1958).
[2] Randall, J. T., *Symp. Soc. Exp. Biol.*, **10**, 185 (1957).
[3] Ris, H., in symposium on the Chemical Basis of Heredity (Johns Hopkins Press, 1957).
[4] Crick, F. H. C., in symposium on the Chemical Basis of Heredity (Johns Hopkins Press, 1957).

Chromosome Numbers of Four Species of the Genus *Dendrocoelum* Œrsted (*Turbellaria tricladida paludicola*)

Cytotaxonomical studies have been made on four species of planarians referred to the genus *Dendrocoelum*, namely, *D. album* (Steinmann), *D. infernale* (Steinmann), *D. lacteum* (O. F. Müller) and *D. tubuliferum* (De Beauchamp). The first and the last species were collected in Yugoslavia; *D. infernale* came from the locality in Switzerland reported by Aeppli[1], while the material of *D. lacteum* originated from Austria, Denmark, Germany, Sweden, Switzerland, and Wales.

The following chromosome numbers were recorded: *D. album*: $2n = 28$, and 14 regular bivalents formed during both oogenesis and spermatogenesis; *D. infernale*: $2n = 32$, and during oogenesis 16 bivalents appeared; the spermatogenesis could not be reliably analysed; *D. lacteum*: in all specimens $2n = 14$ were recorded and 7 bivalents were found in both oogenesis and spermatogenesis; *D. tubuliferum*: $2n = 32$, and 16 bivalents were recorded during both the oogenesis and the spermatogenesis.

The results of this investigation do not support the suggestion of Aeppli[1] that *D. infernale* represents an example of naturally occurring polyploidy and that it is a tetraploid of *D. lacteum*. Though I agree with Aeppli that *D. infernale* has 32 chromosomes, I cannot confirm his statement that the chromosome number of *D. lacteum* is $2n = 16$. In some older papers (for references see Aeppli[1]) it is reported that the haploid chromosome number of *D. lacteum* is 8; but I have found it to be only 7 and that $2n = 14$, the same results as those reported by Gelei[2]. However, the chromosome numbers recorded in the species of *Dendrocoelum* which have been studied indicate that polyploidy might have played a part in their evo-

lution. Apart from Aeppli's paper, polyploidy has not been reported previously in the family Dendrocoelidae. But in another group of fresh-water triclads, the family Planariidae, several examples of naturally occurring polyploidy are known. In some morphologically uniform species of this family a series of forms ('microspecies') exists with different chromosome numbers due to replication of the basic sets. This has occurred to such a degree that enneaploid and decaploid numbers have been recorded[3].

Full details of the cytotaxonomical analyses reviewed above will be given elsewhere in the near future.

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[1] Aeppli, E., *Z. ind. Abst.-Vererb.-lehre*, **84** (3), 182, 1952 (1951).
[2] Gelei, J., *Arch. Zellforsch.*, **11**, 51 (1913).
[3] Dahm, A. G., "Taxonomy and Ecology of Five Species Groups in the Family Planariidae" (Malmö, 1958).

VIROLOGY

Eclipse Phase of Vaccinia Virus

THE development of a plaque technique for titrating vaccinia virus on monolayers of chick embryo cells without an agar overlay[1] has furthered the investigation of cell-virus interaction in this system and has produced evidence of an 'eclipse phase' under certain experimental conditions. The essential prerequisite was that the inoculum, consisting of an elementary body suspension prepared from a rabbit skin-passaged strain of vaccinia by the method of Hoagland, Smadel and Rivers[2], was treated ultrasonically before use. Following the infection of monolayers by contact with this inoculum for 5 min. there was a rapid loss of infectivity of adsorbed virus, estimated to be probably about 30–40 per cent of the amount adsorbed, occurring within 10 min. from the first contact between virus and cells. Thereafter the rate of loss was slower, a further 70 per cent of the virus present at the end of the first 10 min. disappearing during 4–6 hr. The titre of virus in the culture began to rise at 7–8 hr. due to production of new virus.

At the period of lowest infectivity, after about 5 hr. of incubation, monolayers were dispersed by trypsin or ethylenediamine tetraacetic acid. The resulting cell suspensions were titrated as whole cells and after disintegration by ultrasonic treatment. Significantly higher titres were obtained with cells, the ratios being of the order of 15–30 : 1.

It was not possible to assess with confidence the quantitative difference in titre between whole and disintegrated cells since a number of possible fallacies inherent in the experimental methods have been demonstrated. Both trypsin and ethylenediamine tetraacetic acid caused the cells to shed about half their virus, and this was discarded when the cells were washed before titration. This virus would not have been removed by washing the monolayers or released spontaneously into the medium. There is no certainty that all the cells potentially capable of producing new virus would survive and do so when inoculated to another monolayer.

The low titre of disintegrated cells was not due to harming the virus by the ultrasonic treatment used to disintegrate them. Such treatment is an efficient method for releasing virus from cells but there is no way of establishing that all the virus in cells is made detectable by this means. In titrating virus in disintegrated cells a dilution of at least 1 : 8–1 : 16 was required to obtain maximum plaque counts.

Taking these points into consideration, the results obtained appear nevertheless to indicate an eclipse phase as they demonstrate a marked fall in infectivity of a culture before the production of new infective virus and, at the stage of lowest infectivity, that cells which do not contain infective virus will ultimately produce it. The early and rapid loss of a considerable part of the infectivity of a culture, within a few minutes of first contact with virus, suggests that some virus is 'eclipsed' almost at once although the process continues for several hours. This probably indicates asynchrony in the cells of the monolayer. A similar time relationship for the escape of adsorbed virus from neutralization by antiserum has been shown and there is evidence that this escape indicates initiation of infection.

Similar evidence for an eclipse phase was not obtained when the inoculum was used without ultrasonic treatment. With this inoculum no significant loss of infectivity of adsorbed virus could be detected before appearance of new infective virus. A loss of about 70 per cent occurred in 8 hr. if the adsorbed virus was treated with antiserum before incubation and only the unneutralizable portion taken into account. Even so, attempts to demonstrate a difference in titre between whole and disintegrated cell suspensions from cultures of HeLa cells by pock counts at the phase of lowest infectivity were unsuccessful; both had about the same titre.

It appears, therefore, that ultrasonic treatment of an elementary body suspension affected the subsequent interaction between virus and cells in chick embryo cell monolayers. The treated virus was more uniformly capable of infecting cells and going into an eclipse phase. It caused a more rapid production of new virus and a greater yield. Why this should be so is not explained. The titre of an elementary body suspension was increased several times by ultrasonic treatment; and when monolayers that had adsorbed virus from an untreated suspension were disintegrated immediately afterwards, the amount of virus recovered by either plaque or pock titration was up to 10–15 times greater than the number of plaques that formed on similar monolayers incubated undisturbed with all their adsorbed virus. The simplest explanation would be that clumps of elementary bodies were dispersed by the treatment, but attempts to get microscopic evidence of it, by dark ground or electron microscopy, were unsuccessful.

The effect of ultrasonic treatment on virus may depend upon the nature of the virus preparation and its source. Preliminary observations suggest that the titre of a crude preparation of virus made by breaking up infected chorioallantoic membranes and removing the gross debris by centrifugation was not increased by the amount of ultrasonic treatment used for the elementary body suspensions.

A full account of this work will be published in due course.

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[1] Postlethwaite, R., *J. Gen. Microbiol.*, **21**, 7 (1960).
[2] Hoagland, C. L., Smadel, J. E., and Rivers, T. M., *J. Exp. Med.*, **71**, 737 (1940).

Transmission of the Viruses of Trachoma, Inclusion Blennorrhœa and Lymphogranuloma to Mice

Many viruses of the psittacosis-lymphogranuloma group can be transmitted intracerebrally in mice. Recently, we have succeeded in passing some strains of the viruses of trachoma and inclusion blennorrhœa by this route. Most suitable for the purpose are 5-week-old animals from a closed colony originating from the *BSVS* strain[1]. The passages were initiated with infected yolk sac. Cortisone (0·5 mgm. subcutaneously daily, beginning 24 hr. before virus) did not apparently facilitate infection.

Four series of passages, at 5–7 day intervals, with the T'ang strain of trachoma, were all successful.

Definite symptoms of disease first developed after 3–4 passages, and are evident 3–4 days after infection. The fur becomes ruffled and the animals adopt a hunched posture and a stilted, trembling gait. The mice often scratch themselves excessively and may develop severe purulent conjunctivitis. Later the crania of the mice appear swollen due to cerebral oedema.

Neither elementary bodies nor inclusions have yet been detected in brain smears stained with Giemsa–May–Grünwald, the iodine method of Smith, Gilkes and Sowa[2] or by Castañeda's method. Preparations of infected brain made by differential centrifugation or by ether extraction do not appear to contain elementary bodies. However, virus can be readily detected by inoculating infected brain into the chick embryo yolk sac where it induces the formation of the characteristic elementary bodies. Mouse brain virus also induces the formation of inclusion bodies in cell cultures (unpublished observations).

Material from the seventeenth passage produced no reaction when injected intracerebrally into a macaque monkey, or when rubbed on to the lightly abraded conjunctiva of the upper lid of a second monkey.

On a number of occasions, infected brains have been titrated by the intracerebral method in mice and chick embryos. By either method the usual titre is of the order of 10^4 *ID*50/ml.

Suckling mice inoculated with this strain of virus show little sign of infection until 10–14 days later. When titrated in suckling mice the apparent titre of the preparation used was 10^2 *ID*50/ml. after one week, as judged by the proportion of mice showing symptoms at that time. One week later the apparent titre had risen to $10^{2\cdot9}$ *ID*50/ml.

A boiled ether-acetone extracted mouse brain antigen was prepared by Casals's[3] method. This fixed complement in the presence of serum from a patient with lymphogranuloma diluted to 1 in 128, thus demonstrating the presence of the lymphogranuloma-psittacosis group antigen.

At each virus passage normal mouse brain was passed in series as a control. None of the control animals showed evidence of infection, thus eliminating the possibility of latent infection with other viruses of the psittacosis-lymphogranuloma group.

Material from each passage was seeded on to 5 per cent horse blood agar plates and into Brewer's thioglycollate medium. An occasional bacterial contaminant was encountered, but in the great majority of instances no bacterial growth occurred.

Three attempts to carry the brain-adapted virus intraperitoneally in mice were unsuccessful, whether cortisone was given or not. In one series virus was recovered from the spleen after 3 but not after 6 passages at 7-day intervals. In two series virus was recovered only on the first passage. No symptoms of disease developed.

Three series of intracerebral passages with the *G*1 strain of trachoma virus, with and without cortisone, and two series with the *G*17 strain were all negative and virus could not be re-isolated after from 2 to 4 serial passages.

Two series of intracerebral passages with the *LB*2 strain of inclusion blennorrhœa appeared to be succeeding at the first passage, and virus was recovered in the yolk sac. At subsequent passages, the initial mild symptoms of infection failed to be reproduced and passage into eggs was negative.

By contrast, a series of passages with the *LB*1 strain of inclusion blennorrhœa has been carried successfully through 13 serial transfers, with recovery of the virus in the yolk sac at intervals up to the twelfth passage. The yolk sac virus used to initiate infection was more toxic than was the T'ang virus and dilutions to 10^{-3} killed the majority of the recipients within 2–3 days. After intracerebral injection of dilutions of 10^{-3} or 10^{-4}, symptoms of disease appeared on the seventh day at the first passage. They did not differ from those described above.

As far as the mouse is concerned, therefore, the behaviour of some strains of trachoma and inclusion blennorrhœa is very similar to that of the lymphogranuloma virus. Through the courtesy of Prof. C. Barwell, we obtained 2 strains of this virus, one the *J.H.* strain long-adapted to the yolk sac, and the other (Ross) a strain recently isolated from man. On intracerebral passage in mice both behaved similarly to the T'ang virus, and after 3–4 passages produced symptoms indistinguishable from those described above. (It should be added that the viruses used in this investigation have, throughout, been handled in different isolation units, and the various series of transfers have been carried out by different personnel.) Attempts to carry these lymphogranuloma viruses serially by intraperitoneal inoculation of mice failed.

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[1] Webster, L. T., *J. Exp. Med.*, **65**, 261 (1937).
[2] Gilkes, M. J., Smith, C. H., and Sowa, J., *Brit. J. Ophthal.*, **42**, 473 (1958).
[3] Casals, J., Oiticky, P. K., and Anslow, R. O., *J. Exp. Med.*, **94**, 123 (1951).

STATISTICS

Earthquakes and Uranus: Misuse of a Statistical Test of Significance

There are always a large number of persons engaged in searching for correlations between pairs of variables, most of which are, in fact, uncorrelated. From the way in which statistical tests of significance are constructed, we know that in the cases in which no correlation exists the search will be spuriously rewarded, on the average once in every hundred

tests, by a result significant at the 1 per cent level. When interpreting a published result significant at this level, it is necessary to make allowance for the possible existence of ninety-nine other results which were not published because they were not significant.

A spuriously significant result may be obtained in a somewhat similar way when an investigator examines a large body of observational data before deciding exactly what test to apply, or what portion of the data to test. This procedure is equivalent to applying many tests (perhaps thousands) and selecting the most significant result for report.

In a study of the influence of different factors on earthquakes, Tomaschek[1] has found that among the 23 great earthquakes ($M \geqslant 7\frac{3}{4}$) which occurred between December 1903 and December 1906, there were 15 cases in which the earthquake occurred within $\pm$ 1 hr. of the (upper or lower) transit of Uranus across the meridian of the epicentre. For brevity, let us call each earthquake a 'trial', and let us say that a 'success' has occurred if Uranus is found to be within $\pm$ 1 hr. of transit. On the null hypothesis that the times of occurrence of earthquakes are independent of the positions of Uranus, the probability of success in each trial is $p = 1/6$, and the expected number of successes in 23 trials is 23/6. Referring to the 15 successes observed in 23 trials, Tomaschek states: "Application of the chi-square test gives $\chi^2 = 41$; with Yates's correction $\chi^2 = 37$, that is, for one degree of freedom, a probability much less than 0·0001 for its being a chance distribution. The deviation is therefore highly significant".

If the whole study had been confined to the three-year period in question (selected in advance), and if the principal object of the investigation (nominated in advance) had been to test for association between earthquakes and the positions of Uranus throughout this period, then the exact testing procedure (to which the chi-square test is an approximation) would have been to compute the probability $P = {}^{23}C_{15}p^{15}q^8 + {}^{23}C_{16}p^{16}q^7 + \ldots$ of obtaining 15 or more successes in 23 trials, with $p = 1/6$ and $q = 1 - p$. This yields $P = 0{\cdot}269 \times 10^{-6}$, a refinement of Tomaschek's result "much less than 0·0001". Hence, Tomaschek could have claimed significance at the level 10^{-6}, so that his result would have supplied almost overwhelming evidence against the null hypothesis for the three-year period.

But this procedure is invalidated by the facts that: (*a*) not just 23 but 134 earthquakes were investigated; (*b*) temporary associations were looked for, neither the date of commencement nor the duration being specified in advance; (*c*) the positions of the Sun, Moon and other planets, presumably a total of at least ten celestial bodies, were included in the study. By making reasonable assumptions about the class of alternative hypotheses virtually included in the composite test, we can estimate the necessary adjustment to the significance-level as follows.

Consider a series of N ordered trials with constant probability of success $p = 1/6$. If $N = 8$, the probability of obtaining 8 successes is $p^8 = 0{\cdot}595 \times 10^{-6}$, so that this outcome is significant at the level 10^{-6}. If $N = 134$, the probability of obtaining at least one run of 8 or more consecutive successes is very nearly $p^8(1 + 126q) = 63{\cdot}1 \times 10^{-6}$, so that this outcome is no longer significant at the level 10^{-6}; but we suppose that an outcome of this kind would have been reported by Tomaschek as "highly significant" of a temporary increase in p, and we shall call it pseudo-significant at the level 10^{-6}. Similarly, series containing any of the following are pseudo-significant at the level 10^{-6}: 9 or more successes in 10 consecutive trials; 10 or more in 12; 11 or more in 14; 12 or more in 16; 13 or more in 19; and so on.

It would be useful to have a general formula $P = P(N,p,\alpha)$ for the probability P that the outcome of a series of N trials should contain at least one subset pseudo-significant at the level α. As no such formula is available, we set a lower bound to the value of P by a partial enumeration as follows. With $N = 134$ and $\alpha = 10^{-6}$, the contribution to P due to series containing 8 or more consecutive successes is $63{\cdot}1 \times 10^{-6}$. The contribution due to series containing 9 successes in 10 but not 8 in 8 is $49{\cdot}2 \times 10^{-6}$. The contribution due to 10 successes in 12 but not 9 in 10 nor 8 in 8 is $30{\cdot}3 \times 10^{-6}$. The contribution due to 11 successes in 14 but not 10 in 12 nor 9 in 10 nor 8 in 8 is $14{\cdot}6 \times 10^{-6}$. Estimation of further contributions is tedious and has not been carried out, but on adding the four contributions given above we obtain $P > 1{\cdot}57 \times 10^{-4}$.

We now suppose that the observational data comprise 10 series, each of 134 trials. The probability that at least one of these 10 series will contain a pseudo-significant subset when the null hypothesis is true is $1 - (1 - P)^{10} \simeq 10P$. Since $10P > 1{\cdot}57 \times 10^{-3}$, we infer that Tomaschek's result is not significant at the level 10^{-3}, although it may be significant at the level 10^{-2} (that is, the 1 per cent level).

This means that we must choose between two conclusions: either the null hypothesis is to be rejected in favour of the alternative hypothesis of a temporary association between the occurrence of great earthquakes and the positions of Uranus; or we retain the null hypothesis and say that an unlikely coincidence has occurred—a coincidence which would occur (on the average) more often than once in 1,000 investigations, but perhaps less often than once in 100.

In the absence of any theory to account for the supposed association, and in view of the remarks contained in the opening paragraph above, the second conclusion seems preferable.

Finally, we observe that even if we knew the exact significance-level or an exact likelihood ratio, we should not be entitled to make a probability statement about the truth of the null hypothesis, as Tomaschek appears to have done; for no such statement is possible[2] without *a priori* knowledge of the probability of the truth of this hypothesis.

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Jan. 7.

[1] Tomaschek, R., *Nature*, **184**, 177 (1959).
[2] Fisher, R. A., "Statistical Methods and Scientific Inference" (Oliver and Boyd, Edinburgh, 1956).

I ENTIRELY agree with Mr. Burr's statements contained in his first four paragraphs. I stated that this overwhelming evidence belongs to the three years contained in my Table 1. Regarding the total material of the 134 greatest earthquakes which were accurately observed over a period of 49 years (that is, the material contained in Gutenberg-Richter's book) I stated explicitly that "after 1906 the correlation becomes less significant, but it remains greater

than average". In fact, in this complete set of 134 cases of the strongest earthquakes, Uranus was 39 times within ± 1 hr. of the meridian (expected 22·3 times). That is significant at a level below 10^{-3}. This disposes of the starting point of Mr. Burr's criticism.

To state the plain facts: One is led to the conclusion that the position of Uranus within $\pm 15°$ of the meridian at the moment of great earthquakes can be regarded as significant and that there exist times of longer period (several years) when it is very highly significant. It is quite obvious that the strains and stresses in the Earth's crust are the primary cause of earthquakes, but it has been shown, given the presence of these factors of sufficient magnitude, that the timing of the event in a significant number of cases can be described by the position of Uranus. If it is necessary to explain this within the limits of present-day science, attention should be directed to the fact that Uranus is the only planet of which the direction of its axis of rotation coincides with the plane of its orbital revolution. A possible magnetic field would influence the solar plasma in a way quite different from all the other planets.

The earthquake which destroyed Agadir on February 29 this year occurred with Uranus only about 4° from the meridian. Anybody in Agadir, knowing of my communication in *Nature*, and being warned by the preceding minor shocks and the reported behaviour of animals[1], would have kept away from buildings at the time of Uranus being near the meridian, which was from about 10 hr. to 12 hr., a.m. or p.m. The destruction of the town occurred at 11 hr. p.m. local time. An unbiased approach to these problems, of which the correlations of Uranus are only a part and a first step, may help humanity.

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[1] In seismic regions, as for example in Japan, Russia and Italy, sensitive tiltmeters give valuable indications on the actual condition of the Earth's crust (tilt-storms); see Nos. 600–617 of the bibliography of the Commission Permanente des Marées Terrestres (Observatory, Brussels, 1959).

Probability and Statistics

If $k_1(x)$ is any non-negative function in L_1 $(-\infty, +\infty)$, let us write:

$$k_n(x) = \int_{-\infty}^{+\infty} k_{n-1}(x-z)k_1(z)\mathrm{d}z$$

for $n = 2, 3, \ldots$. Then the function:

$$\Delta[k_1(x)] = \sum_{n=1}^{\infty} k_n(x)$$

is defined almost everywhere (although it is possibly infinite for some, or all, x).

Suppose $f_1(x)$ is the frequency function of a random variable X, the mean value of which $\mu_1 = EX$ is strictly positive (it may have the value $+\infty$). Then the renewal density theorem provides conditions on $f_1(x)$ under which, as $x \to \infty$:

$$\Delta[f_1(x)] \to \frac{1}{\mu_1}$$

The function $\Delta[f_1(x)]$ is called the renewal density function, and its limiting behaviour has been an object of study from the earliest days of renewal theory. Prior to the important paper of Feller[1] there appears to have been some controversy concerning this behaviour. Feller provided sufficient conditions under which the renewal density theorem would hold. These conditions have been modified and simplified by Täcklind[2] and Smith[3,4]. The simplest sufficient conditions to date are those given in ref. 4; they are: (i) $f_1(x) \to 0$ as $x \to \infty$; (ii) $f_1(x)\, \varepsilon L_p(-\infty, +\infty)$ for some $p > 1$.

In the course of some work on theory of dams I have recently discovered[5] that if:

$$f_1(x) = \int_0^{\infty} \frac{e^{t-(t+x)}x^{t-1}}{\Gamma(t)}\mathrm{d}t, \quad x > 0$$

$$= 0, \quad x < 0$$

then $\mu_1 = 1$ and $\Delta[f_1(x)] \to 1$, that is, the renewal density theorem holds. However, $f_1(x)$ is in no class L_p for $p > 1$. Thus the conditions of ref. 4 are not necessary.

We have now proved that the following constitute necessary and sufficient conditions for the validity of the renewal density theorem. They are:

$$f_1(x) \to 0 \text{ as } x \to \infty \tag{1}$$

For every (small) $\delta > 0$, if

$$a_\delta(x) = f_1(x) \text{ for } 0 < x < \delta,$$
$$= 0, \text{ otherwise,}$$
$$\text{then } \Delta\,[a_\delta(x)] \to 0 \text{ as } x \to \infty \tag{2}$$

For every (small) $\delta > 0$, if:

$$\varphi_\delta(x) = \int_\delta^{\infty} e^{ixy} f_1(y)\mathrm{d}y$$

then $\varphi_\delta(x)$ belongs to some class L_p $(p > 0)$, where p may depend upon δ. (3)

This is the first time that necessary and sufficient conditions for this theorem have been established. It will be seen that they are substantially less restrictive than the sufficient conditions of ref. 4; for example, no restraint is placed on the behaviour of $f_1(x)$ for negative x.

Condition (2) does not seem easy to verify; but we have shown that it is satisfied if $f_1(x) = 0(x^{-N})$ in $(0,\delta)$ for some sufficiently large N; in particular, it is satisfied if $f_1(x)$ is monotone in $(0,\delta)$. Condition (3) is satisfied if $f_1(x)$ belongs to $L_p(\delta,\infty)$ for every $\delta > 0$, where p may depend upon δ, but must exceed unity.

The motivation for our conditions is that only the behaviour of $f_1(x)$ in the open interval $(0,\infty)$ should affect the behaviour of $\Delta[f_1(x)]$ for large x. Singularities of $f_1(x)$ at the origin should remain at the origin for $f_n(x)$ and not be displaced to larger values of x. Thus, for large values of x, $\Delta[f_1(x)]$ should not be affected by singularities of $f_1(x)$ at the origin.

Needless to say, the 'counter-example' quoted above satisfies the new necessary and sufficient conditions.

I intend to publish full details of the proof in the near future.

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[1] Feller, *Ann. Math. Statist.*, **12**, 243 (1941).
[2] Täcklind, *Skand. Akt.*, **28**, 68 (1948).
[3] Smith, *Proc. Roy. Soc. Edin.*, **64**, 9 (1954).
[4] Smith, *Proc. Camb. Phil. Soc.*, **51**, 629 (1955).
[5] Smith, *Hotelling Festschrift* (in the press).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, April 25

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. C. W. Phillips: "The Ordnance Survey and Archæology".

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. Devons: "Applied Economics: Science, Wisdom, Hunch or Witchcraft?"

SOCIETY OF CHEMICAL INDUSTRY, SURFACE ACTIVITY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Annual General Meeting, followed by Sir Harry Melville: "Ion Exchange Resins and their Application".

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "Anglo-American Comparisons of Productivity".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. K. C. Kao: "A Survey of Microwave Techniques for the Transmission of Speech and Vision".

Monday, April 25—Friday, April 29

ADVISORY GROUP FOR AERONAUTICAL RESEARCH AND DEVELOPMENT, WIND TUNNEL AND MODEL TESTING PANEL, NORTH ATLANTIC TREATY ORGANIZATION ((at Whitehall Gardens, London, S.W.1)—Meeting on "Boundary Layer Research".

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (at Church House, Westminster, London, S.W.1)—International Meeting on Veterinary Education.

INTERNATIONAL COMPRESSED AIR AND HYDRAULICS EXHIBITION (at Olympia, London).*

Tuesday, April 26

INSTITUTION OF INDUSTRIAL SAFETY OFFICERS (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. A. G. Palmer: "Design and Use of Clearance Certificate Systems" (Second Alexander Redgrave Memorial Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. T. C. Cardwell, Mr. J. R. W. Smith and Mr. G. H. King: "An Experimental Transistor-Controlled Component Selection and Testing Machine".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. T. E. Barany: "Some Recent Advances in Industrial Electrical Control Techniques".

Wednesday, April 27

BRITISH ASSOCIATION FOR COMMERCIAL AND INDUSTRIAL EDUCATION (at the Federation of British Industries, 21 Tothill Street, London, S.W.1), at 11 a.m.—Conference on "The Implications of the Crowther Report".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 3 p.m.—Annual General Meeting.

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Discussion on "The Present Position of Econometrics" opened by Mrs. Joan Robinson, Mr. J. S. Downie and Mr. C. B. Winsten.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. Chaste and Mr. P. Cassagne: "Henri de France Colour Television System".

BRITISH INSTITUTION OF RADIO ENGINEERS, LONDON SECTION (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. M. J. Tucker: "Electronics in Oceanography".

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. Washburn (University of California): "Deformation and Fracture of Ionic Crystals".

INSTITUTION OF WOOD SCIENCE (at Imperial College, Exhibition Road, London, S.W.7), at 6.45 p.m.—Prof. R. D. Preston: "The Sub-Microscopic Structure of Wood".

Wednesday, April 27—Friday, May 6

FUEL EFFICIENCY AND POWER FOR INDUSTRY EXHIBITION (at Olympia, London).*

Thursday, April 28

BRITISH COMPUTER SOCIETY, LONDON BRANCH (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 2.30 p.m.—Mr. Stafford Beer: "Automation and Uncertainty".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. F. P. Bowden, F.R.S., Mr. J. A. Greenwood, Mr. J. Harris and Mr. J. B. P. Williamson: "Electrical Conduction in Solids: Temperature-dependent Conductors and the Contact of Solids"; Mr. L. C. F. Blackman, Mr. P. H. Dundas and Prof. A. R. Ubbelohde, F.R.S.: "The Anisotropic Thermo-electric Power of Graphite".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. E. Eastwood: "Radar Observations of Birds and 'Angels' ".

INSTITUTE OF INFORMATION SCIENTISTS (joint meeting with the PRESENTATION OF TECHNICAL INFORMATION GROUP, at the College of Preceptors, 2 Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Discussion on "Scientists, Information Officers and This Writing Business" opened by Dr. B. C. Christian and Mr. J. B. Reed.

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting.

BRITISH INSTITUTION OF RADIO ENGINEERS, LONDON SECTION (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Discussion on the Education Committee's Report on "The Education and Training of the Professional Radio and Electronics Engineer".

BRITISH INTERPLANETARY SOCIETY (in the Hoare Memorial Hall, Church House, London, S.W.1), at 7 p.m.—Dr. E. W. Still: "High Altitude Chambers and Pressure Suits".

Friday, April 29

FARADAY SOCIETY (in the Large Chemistry Lecture Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. H. J. Bernstein (National Research Council, Canada): "Photoelectrically Recorded Raman Spectra of Some Gases" (Bourke Lecture).

SCIENTIFIC PUBLICATIONS COUNCIL (at the Ciba Foundation, 41 Portland Place, London, W.1), at 5.45 p.m.—Discussion Meeting on "Editorial Influence" introduced by Dr. R. D. Keynes, F.R.S.

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Annual General Meeting, followed by Dr. J. D. Kendall: Chairman's Address.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with an honours degree and preferably some teaching and/or industrial experience) IN MATHEMATICS (full-time) AT THE COLLEGE OF TECHNOLOGY, Byrom Street, Liverpool, to teach to graduate level—The Director of Education, City of Liverpool Education Committee, 14 Sir Thomas Street, Liverpool 1 (April 27).

CHEMISTS (Senior Scientific Assistant or Scientific Assistant, Class 1 (with at least a first- or second-class honours special degree in chemistry, or its equivalent, and extensive experience in chemical analysis and in the investigation of miscellaneous problems of a chemical nature) at the Central Laboratory, Chiswick, which deals with problems relating to a wide variety of materials including fuels and lubricants, detergents, water and effluents, rubber and plastics, textiles, etc.—Staff and Welfare Officer (F/EV. 7864), London Transport, 55 Broadway, London, S.W.1 (April 30).

LECTURER IN INORGANIC CHEMISTRY—The Registrar, The University, Manchester 13 (April 30).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Secretary, The University, Exeter (April 30).

SENIOR LECTURER IN MATHEMATICS; a LECTURER IN MATHEMATICS to teach degree and final professional level; and an ASSISTANT (Grade "B") IN MATHEMATICS to teach up to G.C.E. ordinary level and H.N.C.—The Principal, Derby and District College of Technology, Kedleston Road, Derby (April 30).

TECHNICIAN (preferably A.I.M.L.T.) TO THE SURGICAL PROFESSORIAL UNIT—The Secretary, Charing Cross Hospital Medical School, 62–65 Chandos Place, London, W.C.2 (April 30).

SENIOR LECTURER or LECTURER (honours graduate with appropriate teaching experience) IN PURE MATHEMATICS; and a SENIOR LECTURER or LECTURER (honours graduate with appropriate teaching experience) IN STATISTICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (May 2).

STATISTICIAN (with a degree in statistics and/or mathematics or in science, engineering or agricultural science, and preferably with experience of modern statistical techniques)—The Director, An Foras Talúntais (The Agricultural Institute of Ireland), 33 Merrion Square, Dublin (May 2).

ASSISTANT IN PHYSICAL OR INORGANIC CHEMISTRY—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (May 7).

ASSISTANT LECTURER or a LECTURER IN ORGANIC CHEMISTRY—The Registrar, University College, Singleton Park, Swansea (May 7).

ASSISTANT LECTURERS (2) (Veterinary Surgeons, or with a degree in an appropriate biological subject) IN VETERINARY PATHOLOGY; and an ASSISTANT LECTURER IN CHEMISTRY tenable in the Department of Physiology—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (May 7).

SENIOR RESEARCH STUDENT IN EITHER ENGINEERING OR CHEMICAL ENGINEERING OR METALLURGY—The Registrar, University College, Singleton Park, Swansea (May 7).

ASSISTANT LECTURER or LECTURER IN (*a*) ORGANIC CHEMISTRY and (*b*) INORGANIC AND PHYSICAL CHEMISTRY—The Registrar, University College of North Wales, Bangor, North Wales (May 16).

CHEMIST (B.Sc. or A.R.I.C.), for interesting and varied investigations in the User Laboratory—The Director, British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, N.7 (May 16).

CHAIR OF BACTERIOLOGY at University College Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 23).

LECTURER IN PHYSICS—The Registrar (Room 22, O.R.B.), The University, Reading (May 23).

LECTURER IN PHYSIOLOGY—The Secretary, The University, Aberdeen (May 23).

READER IN ANATOMY at St. Bartholomew's Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 26).

FELLOW or SENIOR RESEARCH FELLOW, to work in the field of electrophysiology of the central nervous system; a RESEARCH FELLOW, to work in the field of neuropharmacology; and a FELLOW to carry out fundamental research on the synthesis and the kinetics of substitution reactions of co-ordination compounds of the heavier transitional elements, at the John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 27).

AFRICAN EXPLOSIVES AND CHEMICAL INDUSTRIES LIMITED CHAIR OF CHEMICAL ENGINEERING at the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, May 31).

PROFESSOR OF MATHEMATICS OR APPLIED MATHEMATICS at the University of Natal, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, May 31).

RESEARCH FELLOWS (2) and a SENIOR RESEARCH FELLOW IN THE DEPARTMENT OF NUCLEAR PHYSICS, Research School of Physical Sciences, Australian National University, to work either in the field of nuclear spectroscopy or on photonuclear problems—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (May 31).

CHAIR OF VETERINARY PATHOLOGY at the Royal Veterinary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (June 1).

LECTURER/SENIOR LECTURER IN BIOCHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 3).

ASSISTANT LECTURER, Grade B (graduate with teaching and industrial experience), IN THE SCHOOL OF PHYSICS AND APPLIED SCIENCE—The Registrar, College of Technology and Commerce, Leicester.

ASSOCIATE or ASSISTANT PROFESSOR IN THE DEPARTMENT OF PHARMACOLOGY, to assist in the teaching of pharmacology to medical and dental students, with excellent facilities and opportunity for individual research—Prof. K. I. Melville, Chairman, Department of Pharmacology, McGill University, Montreal, P.Q., Canada.

BIOCHEMIST (biochemistry, chemistry or physiology graduate) IN THE PATHOLOGICAL LABORATORY—The Group Secretary or the Pathologist, Dulwich Hospital, East Dulwich Grove, London, S.E.22.

BIOCHEMIST, Senior grade, to assist in research mainly in the corticosteroid field—The Group Secretary, Sheffield No. 1 Hospital Management Committee, Nether Edge Hospital, Sheffield 11.

BIOCHEMIST (Senior or Basic grade) to the Rochdale Group of Hospitals, to work under the Consultant Pathologist and to carry out biochemical work for the hospitals and clinics of the Group—The Group Secretary, Rochdale and District Hospital Management Committee, Central Offices, Birch Hill Hospital, Rochdale, Lancs.

ELECTRONICS TECHNICIAN (capable of constructing and designing electronic apparatus of unconventional types as applied to astronomical observation) IN THE DEPARTMENT OF ASTRONOMY—The Professor of Astronomy, The University of Manchester, Oxford Road, Manchester 13.

GRADUATE RESEARCH ASSISTANT IN THE DEPARTMENT OF TROPICAL MEDICINE, for biological medical research—The Dean, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool 3.

ISOTOPE TECHNICIAN (preferably with a knowledge of biochemistry, electronics or radiography), to work in a specialized laboratory dealing with routine and research investigation in thyroid disorders—The Secretary, New End Hospital, Hampstead, London, N.W.3.

RESEARCH ASSISTANT (with a first- or second-class honours B.Sc. (Special or General)) IN PHYSICAL BIOCHEMISTRY—The Principal, South-West Essex Technical College and School of Art, Forest Road, Walthamstow, London, E.17.

RESEARCH ASSISTANT (with a first- or second-class honours degree in biochemistry), to assist with investigations into disturbances of calcium metabolism—The Director, Metabolic Disturbances in Surgery Research Unit, The General Infirmary, Leeds.

SCIENCE GRADUATE (preferably with biological training), to work as a member of a team studying the haematological problems of cardiac surgery and coronary heart disease—Dr. A. H. T. Robb-Smith, Department of Pathology, Radcliffe Infirmary, Oxford.

SCIENTIFIC OFFICER (MEDICAL) (with an honours degree in science (biochemistry) of a British University and one years postgraduate experience in a hospital clinical biochemistry laboratory) in Hong Kong, for work in the Biochemistry Section of the Government Institute of Pathology, and for duties connected with the training of a technologist—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.117/51/056.

TECHNICIAN IN THE NUFFIELD DEPARTMENT OF SURGERY, University of Oxford, for work which will entail the care of electronic equipment, artificial heart pumps and assistance in the use of these—The Nuffield Professor of Surgery, The Radcliffe Infirmary, Oxford.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Education. The Youth Service in England and Wales: Report of the Committee Appointed by the Minister of Education in November, 1958. Pp. iv+136. (Cmnd. 929.) (London: H.M. Stationery Office, 1960.) 6s. net. [92

Institution of Electrical Engineers. List of Members, Part 1: Members, Associate Members and Companions. Pp. 604. (London: Institution of Electrical Engineers, 1959.) [172

Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1958–59. Aden Protectorate. Pp. 25. 1s. Uganda. Pp. 75. 1s. Republic of the Sudan. Pp. 35. 1s. (London: Empire Cotton Growing Corporation, 1960.) [172

The West of Scotland Agricultural College. Comparison of Production from six Cultivars of Timothy, 1954–1957. By I. V. Hunt. (Research Bulletin No. 27.) Pp. 39. (Auchincruive, by Ayr: West of Scotland Agricultural College, 1959.) 1s. [172

Parliamentary and Scientific Committee. Annual Report, 1959. Pp. 28. (London: Parliamentary and Scientific Committee, 1960.) [172

Natural Rubber Development Board. British Rubber Producers' Research Association Technical Bulletin No. 4: Compounding Natural Rubber for Service at Low Temperatures. By S. G. Fogg and P. M. Swift. Pp. 26. (London: Natural Rubber Development Board, 1960.) [172

Other Countries

Berichte des Deutschen Wetterdienstes. Nr. 58 (Band 8): Über die Eiskeimbildung in unterkühlten Wolken in ihrer Abhängigkeit von Atmosphärischen Aerosol. Von Hans-Walter Georgii. Pp. 43. 7 D.M. Nr. 59 (Band 8): Alexander von Humboldt-Gedenkfeier in Frankfurt a. M. am 19 Juni 1959. Pp. 31. 5 D.M. Nr. 60 (Band 9): Beitr ge zur Phänologie Spaniens auf Grund der Phänologischen Beobachtungen des Spanischen Wetterdienstes 1943–1954. Von Dietrich Ottmar. Pp. 27. (Offenbach a. M.: Deutschen Wetterdienstes, 1959.) [42

Bulletin of the Bingham Oceanographic Collection, Peabody Museum of Natural History, Yale University. Vol. 17, Article 1: Oceanography of Long Island Sound. By Gordon A. Riley, Eugene Harris, Henry M. Schurr, P. J. Wangersky, Sarah W. Richards and R. Wade Covill. Guide for Preparing Figures. By Y. H. Olsen and James E. Morrow, Jr. Pp. 153. (New Haven, Conn.: Peabody Museum of Natural History, Yale University, 1959.) 3.50 dollars. [42

Smithsonian Institution. Report of the Secretary and Financial Report of the Executive Committee of the Board of Regents for the year ended June 30, 1959. (Smithsonian Publication No. 4389.) Pp. x+243+10 plates. (Washington, D.C.: Government Printing Office, 1959.) [162

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 16, No. 1 (January 1960): Documents Behind the Population Controversy. Pp. 24. (Washington, D.C.: Population Reference Bureau, Inc., 1960.) 50 cents. [162

United States Department of Commerce: Weather Bureau. Technical Paper No. 36: North Atlantic Tropical Cyclones—Tracks and Frequencies of Hurricanes and Tropical Storms, 1886–1958. By George W. Cry, William H. Haggard and Hugh S. White. Pp. iv+214. 1 dollar. Technical Paper No. 37: Evaporation Maps for the United States. By M. A. Kohler, T. J. Nordenson and D. R. Baker. Pp. i+13+5 plates. 65 cents. (Washington, D.C.: Government Printing Office, 1959.) [162

United States Department of Commerce: Coast and Geodetic Survey. Technical Bulletin No. 6: The Tsunami of March 9, 1957, as Recorded at Tide Stations. By Garrett G. Salsman. Pp. ii+18. (Washington, D.C.: Government Printing Office, 1959.) 15 cents. [162

Canada: Department of Mines and Technical Surveys. Publications of the Dominion Astrophysical Observatory, Victoria, B.C. Vol. 11, No. 2: The Chromospheric K-Line of Ca II in the Spectrum of 31 Cygni. By A. McKellar, L. H. Aller, G. J. Odgers and E. H. Richardson. Pp. 35–58. 25 cents. Vol. 11, No. 3: The B-Type Secondary Spectrum and Atmospheric Effects Observed at the 1951 Eclipse of 31 Cygni. By K. O. Wright and E. K. Lee. Pp. 59–75. 25 cents. Vol. 11, No. 5: A Wave-Length Study of the Spectrum of H. F. 188C01, 9 Sagittae. By Anne B. Underhill. Pp. 143–184. 25 cents. (Ottawa: Queen's Printer, 1959.) [172

New Zealand. Report of the Board of Trustees of the National Art Gallery and Dominion Museum for the year ended 31 March 1959. Pp. 28. (H.21.) (Wellington: Government Printer, 1959.) [172

Bulletin of the Madras Government Museum (New Series). Natural History Section, Vol. 6, No. 3: The Amphipoda of the Madras Coast. By K. Nagappan Nayar. Pp. iv+59+16 plates. (Madras: Government Museum, 1959.) Rs. 4.35 nP. [172

India. Forest Research Institute and Colleges, Dehra Dun. Forest Research in India, 1952–53. Part 1: The Forest Research Institute. Pp. ii+149. (Delhi: Manager of Publications, 1959.) Rs. 3.50 nP. [172

Annals of the New York Academy of Sciences. Vol. 79, Article 10: Biology of the Pleuropneumonialike Organisms. By D. G. ff. Edward and 80 other authors. Pp. 805–758. (New York: New York Academy of Sciences, 1960.) 5 dollars. [172

The United States National Museum. Annual Report for the year ended June 30, 1959. Pp. vi+179. (Washington, D.C.: Government Printing Office, 1959.) [172

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